

What is the potential role of veterinary vaccination in the control of Rift Valley fever in Kenya?



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A thesis submitted for the degree of
Doctor of Philosophy

Trinity Term 2020

50,000 (excluding references and appendices)

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Acknowledgements

Working on a veterinary clinical trial across four institutes and two countries has not only produced this thesis as an output but also some extraordinary experiences, an appreciation for the Kenyan culture and land and the deep gratitude for the very talented colleagues I have.

My most sincere thanks go to my DPhil supervisors, George Warimwe, Katie Ewer and Simon Gubbins. To George, who gave me an incredible opportunity to lead this vaccine field trial. To Katie who has always been a beacon of support at Oxford and to Simon who has guided me through the learning experience of understanding mathematical models with the upmost patience. I am grateful to them all for their support and guidance. I am also sincerely grateful to Bryan Charleston who has always provided me with academic and career advice.

A huge thank you to the RVF dream team. To Ilona Gluecks and Annie Cook who have become mentors in the field to me. Asantini to Nelson '*Elephants*' and Sospeter '*Toto – Alsevers*' for their veterinary expertise, Sheillah and Christine for their field-lab processing skills. I am indebted to the Kapiti animal technicians and animal herders and to Nehemiah, John and Kigo for making sure the logistics and security of the farm ran smoothly. A special thank you to Ivan, Tom and the Mpala research centre for letting us sample animals from the Laikipia ranches and to Professor Thumbi and his lab for the use of their samples, time and hospitality in Kisumu.

I gratefully acknowledge John Gitonga, who runs the Kilifi lab meticulously, and who optimised and conducted the FRNT assays for the field trial samples. To Henry, Jackline, Naomi, Stanley, Stephen, Clinton and the rest of the Warimwe lab at KEMRI-Wellcome Trust for your support throughout the field trial and DPhil. It was also an honour to work

with Judy, Irene and Esther in the KEMRI-Wellcome Trust Clinical Trials team and Nick and Jane as the field trial statistical support team at ILRI.

Many thanks to Danny Wright who developed many of the lab assays for the trial and supported me in my journey back into the lab. Thanks to Nick Edwards, the master of anti-vector neutralising antibody assays at the Jenner Institute. I would particularly like to acknowledge Professor Teresa Lambe, Dr Pip Beard and Professor Deirdre Hollingsworth for their expert advice and supportive atmosphere during my confirmation viva.

To the Pirbright Plowright West-Wing '*Aunties*': Bea and Georgina, the go-to R experts and veterinary epi expertise and to Eva, Hannah, Yvonne, Anne and Karin who supported me throughout the write up. Thank you to Nick Lyons for his advice on vaccination evaluation and Antonello di Nardo for advice on graphics. Thank you to the Pirbright Transmission Biology group for always being a place to sound ideas and practice presentations.

A special thanks to Etienne and Santie de Villiers who took me into their home in Kenya and made me a part of their family and to Catherine, the Kapiti guesthouse housekeeper and her son Derick. Thank you to Richard Thompson for his time proof-reading this thesis and to Margaret Thompson for her strength and support. To my mum and dad who have always been my biggest champions and whose own field-trial stories were my dreams and to my sister, El who has always empowered me to do what I love.

My deepest acknowledgements go to David. Your love, support and letters throughout these four years have got me here. May we stop being tumbleweeds tumbling in and out of each other's lives and never have to hear a Whatsapp reconnection dial-tone again!

What is the potential role of veterinary vaccination in the control of Rift Valley fever in Kenya?

Madeleine Harriet Alice Clark, Linacre College, University of Oxford

Thesis submitted for the degree of Doctor of Philosophy, Trinity term 2020

Abstract

Rift Valley fever virus (RVFV) is a zoonotic arbovirus that causes fever, high mortality in young and spontaneous abortions in livestock species. It can have a dramatic impact on livestock health and production as well as on public health and people's livelihoods. The licensed veterinary vaccines have multiple issues including residual virulence, which underlies their contraindication for use in pregnant animals, the need for high containment facilities for their manufacture and diagnostic tests are unable to differentiate between infected and vaccinated animals (DIVA).

The literature indicated that RVF seroprevalence across the African continent was significantly higher in small ruminants during outbreaks than interepidemic periods (IEPs), suggesting these populations are often naïve when an outbreak occurs. The serosurvey conducted in three Kenyan counties found that RVF seroprevalence was recorded in at-risk areas during IEPs, suggesting silent circulation.

A non-inferiority trial was conducted comparing a promising candidate vaccine, ChAdOx1 RVF, with the commercialised Smithburn vaccine in multiple target livestock species (n=613) under natural environment conditions. The successful conduct of the trial in accordance with veterinary GCP has been underpinned by engagement and collaborations with regulatory authorities and trial monitoring teams across multiple sites in Kenya and abroad. The primary endpoint for the trial was to assess non-inferiority of the ChAdOx1 RVF vaccine compared to the Smithburn vaccine with regards to seroconversion as measured by an RVF virus neutralising antibody assay (FRNT). These seroconversion results were utilised in the development of a transmission dynamics model.

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The ChAdOx1 RVF vaccine was found to be safe, DIVA compliant and non-inferior in all species. Vaccine scenarios suggested vaccinating with ChAdOx1 RVF in the dry season annually or every 3 years would decrease cumulative incidence of infected livestock significantly. These results show ChAdOx1 RVF is a promising candidate for registration and use in livestock in areas of risk for RVF.

Statement of originality

I confirm that the work recorded in this thesis is entirely my own, other than the exceptions listed below.

Chapter 1: Introduction

I wrote the introduction. George Warimwe, Simon Gubbins and Katie Ewer reviewed the chapter providing useful comments and suggestions.

Chapter 2: Seroprevalence of Rift Valley fever virus in Africa: a systematic review

I carried out and wrote the systematic review which was published as a paper in PLoS Neglected Tropical Medicine (12(7), e0006627). Antonello di Nardo helped me produce the maps in figure 2.2 and figure 2.4. Simon Gubbins, George Warimwe and Nick Lyons provided advice and comments on early drafts of the paper and the chapter itself. I also received comments from three anonymous journal reviewers when submitting the paper. Katie Ewer also provided useful comments on the chapter.

Chapter 3: Seroprevalence of Rift Valley fever virus in Kenya.

Samples for the Kisumu analysis were provided from a biobank of samples that had already been collected from the KEMRI-CDC programme in Kisumu. I co-ordinated the sampling in Laikipia and Machakos, my colleagues Annie Cook, Ilona Gluecks and Nelson Kipchirchir sampled the animals as Kenyan registered veterinarians. I, along with the veterinarians processed the samples. I conducted the ELISAs, analysed and wrote the chapter. Simon Gubbins, George Warimwe and Katie Ewer all provided helpful comments on the draft chapter.

Chapter 4: Immunogenicity of a Chimpanzee Adenovirus Viral Vected Rift Valley fever vaccine

Livestock samples were already provided from previous vaccine trials at The Pirbright Institute and the Institute of Livestock Research (ILRI). I conducted the anti-vector neutralising antibody assays, analysed and wrote the chapter. Nick Edwards at the Jenner Institute, University of Oxford provided guidance on conducting the neutralisation assays. My colleague Danny Wright completed the RVF Gn ELISAs at the Jenner Institute. Katie Ewer and George Warimwe provided helpful comments on drafts of the chapter.

Chapters 5-7: Non-inferiority vaccine trial

The non-inferiority Rift Valley fever vaccine field trial discussed in Chapter 5, 6 and 7 was conducted with the help of a number of skilled individuals from multiple institutes. I coordinated the trial and was the main point of contact between institutes: I developed the study protocol with George Warimwe, Byran Charleston and Michael Francis and made sure this was implemented correctly during the trial. The veterinarians (Ilona Gluecks, Annie Cook and Nelson Kipchirchir) examined all the animals for clinical and injection site observations as well as took the rectal temperatures. I recorded all the safety data in the field (Chapter 6) along with my colleagues Sospeter Mwata, Sheillah Orono and Christine Mutsiya. I cleaned the dataset, analysed and wrote the chapter. My colleagues at KEMRI-Wellcome Trust Stephen Mwangala, Clinton Mwatata and Stanley Cheruyiot uploaded the hardcopy data collection forms to the electronic Epicollect database which produced the dataset that I cleaned. I conducted the ID Vet ELISAs and helped optimise the focus reduction neutralisation test (FRNT) assay. My colleague John Gitonga at KEMRI-Wellcome Trust carried out the definitive FRNT using the field trial samples. The ILRI statistician, Jane Poole, recoded the data for me so I could analyse the safety results (chapter 6) and the immunogenicity results (chapter 7). I wrote chapter 5, 6 and 7 and these were

reviewed by George Warimwe, Katie Ewer and Simon Gubbins who provided useful comments.

Chapter 8: Modelling the effect of vaccination on RVFV transmission dynamics

The model structure was developed from a previous bluetongue virus transmission model developed by Simon Gubbins. I sought parameters from the literature and adapted the model to fit with RVFV transmission dynamics, I processed the outputs and wrote the chapter. Simon Gubbins provided advice and guidance on coding and analysing the outputs of the model. Simon Gubbins, George Warimwe and Katie Ewer all provided useful comments on the chapter.

Chapter 9: Discussion

I wrote the discussion, and this was reviewed by Katie Ewer, George Warimwe and Simon Gubbins.

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List of Abbreviations

ARRIVE	Animal Research: Reporting of <i>In Vivo</i> Experiments
ChAd	Chimpanzee Adenovirus
CFT	Confined Field Trial
CDC	Centers for Disease Control
DIVA	Differentiating infected from vaccinated animals
DNA	Deoxyribonucleic acid
DVS	Directorate of Veterinary Services
EAC	East African Community
EDTA	Ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ENSO	El Niño Southern Oscillation
FAO	Food and Agriculture Organisation of the United Nations
FDA	U.S. Food and Drug Administration
FRNT	Focus Reduction Neutralisation Test
GCLP	Good Clinical Laboratory Practice
GDP	Gross Domestic Product
GMO	Genetically Modified Organism
IEP	Interepidemic period
IgG	Immunoglobulin G
ILRI	International Livestock Research Institute
KEVIVAPI	Kenya Veterinary Vaccines Production Institute
MCF	Malignant Catarrhal Fever
NBA	National Biosafety Authority

NDVI	Normalized Difference Vegetation Index
OIE	World Organisation for Animal Health
PBASS	Population-based animal syndromic surveillance system
PBIDS	Population-based infectious disease surveillance platform
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase chain reaction
PFU	Plaque-forming unit
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.
PRNTs	Plaque reduction neutralisation test
RNA	Ribonucleic acid
RVF	Rift Valley fever
RVFV	Rift Valley Fever Virus
SOPs	Standard Operating Procedures
SST	Sea Surface temperature
STROBE	Strengthening the Reporting of Observational studies in Epidemiology
TMF	Trial Master File
vGCP	Veterinary Good Clinical Practice
VNTS	Viral Neutralisation Test
WHO	World Health Organisation

1. Introduction

1.1. Background

This doctoral thesis describes my seroepidemiological analyses into the Rift Valley fever virus (RVFV) in Africa, specifically within Kenya, assessing immune responses in livestock species following Rift Valley Fever (RVF) vaccination and the impact livestock vaccination has on RVF transmission dynamics. This research was part of a larger grant funded by the UK government to fast track research and development into vaccines for priority outbreak pathogens following the 2013-2016 Ebola epidemic in West Africa.

A large component of my investigations has been to design, implement and analyse a non-inferiority RVF vaccine field trial assessing immunogenicity and safety in multiple livestock species in Kenya conducted under the principles of veterinary Good Clinical Practice (vGCP). The vaccine immunogenicity results from this trial have been used in the development of a multi-host transmission dynamics model for RVF. The aims of this thesis are: (i) to analyse RVF seroprevalence in Africa; (ii) to estimate non-inferiority, immunogenicity and safety of a novel RVF vaccine in multiple livestock species; and (iii) to understand what impact livestock vaccination has on RVF transmission dynamics. Investigating these aims helps uncover the potential role of RVF vaccination as a control measure in Kenya.

1.2. Rift Valley fever

RVF is a zoonotic arbovirus caused by the Rift Valley fever *Phlebovirus* from the *Phenuiviridae* family and *Bunyavirales* order (Adams et al 2017). Other viruses from the *Phenuiviridae* family include the Sandfly fever virus (SFV) (which encompasses sandfly Naples virus, sandfly Sicilian virus and Toscana virus), as well as Severe Fever with Thrombocytopenia Syndrome virus (SFTSV). RVFV has the ability to infect multiple livestock species, including but not limited to, sheep, goats, cattle and camels and can also

infect humans (Pepin et al. 2010). Wildlife species can seroconvert when exposed and are also considered to play a role in virus maintenance and transmission. Multiple mosquito species can also transmit the virus (Sang et al. 2010). There has been very little research assessing transmission from mosquitoes to humans (Tantely et al. 2015), and the main route of transmission is thought to be through contact with blood and tissue from infected livestock (Anyangu et al. 2010). The disease can cause devastating effects in veterinary and public health. The disease has been identified by the World Health Organisation (WHO) as a priority for research and development for preparedness and response to public health emergencies due to highly infectious pathogens (World Health Organisation 2016).

1.3. RVF pathogenesis

The incubation period and clinical signs are dependent on the inoculation dose, the virus strain, route of infection as well as the age and species of the animal (Easterday, 1965). The incubation period typically ranges from 2-4 days in livestock (Ruiz et al. 2017). Clinical signs in livestock can include fever, nasal and ocular discharges, diarrhoea, vomiting and jaundice (Munyua et al. 2010). Young animals have a much higher mortality than older animals and small ruminants are more susceptible than cattle or camels (Easterday, 1965). Neonatal mortality and abortions are significantly associated with the disease, predominantly in sheep and goats, where they are described as ‘abortion storms’ (Daubney et al. 1931). Infected animals have a high viremia which can range from 1.0×10^6 to 1.0×10^8 plaque forming units (PFUs/mL) and can last for 10 days (Bird et al. 2009). Case fatality rates for young lambs can reach 90-100%, whereas adult sheep have a much lower, but still substantial case fatality rate of 20% (Easterday et al. 1962). Adult cattle are more resistant to the virus and the case fatality rate is approximately 5-10%. It has been suggested that host genetics may play a role in susceptibility as well (Ikegami and Makino, 2011).

The incubation period in humans can range from 4-6 days (Ruiz et al. 2017). Ikegami and Makino (2011) provide a detailed account of the pathogenesis of RVFV in humans. Most humans that become infected have a self-limiting fever which can include weakness, anorexia, dizziness, severe headache, nausea, chills followed by an elevated body temperature, decreased blood pressure, shoulder and backpain among other non-specific symptoms (Ikegami and Makino, 2011). A smaller proportion of cases can go on to cause neurological disorders such as encephalitis (Maar et al. 1971); vision loss including retinopathy, thrombosis, liver necrosis and haemorrhaging leading to fatalities (Ikegami and Makino, 2011). During the 2000 outbreak in Saudi Arabia the case fatality rate in humans reached 14% (Madani et al. 2003). A cross-sectional study has identified that RVF infection is significantly linked with miscarriages in women in Sudan (Baudin et al. 2016). Vertical and transplacental transmission has also been described (Adam and Karsany, 2008, Arishi et al. 2006).

1.4. Diagnostics

The identification of RVF seroconversion is conducted by ELISA or viral neutralisation tests (VNTs). VNTs are the gold standard test as they are highly sensitive and specific. However, ELISA is the most widely used technique due to the ease of use, speed and high containment laboratories are not needed (OIE, 2018). To conduct VNTs a category 3 level laboratory is needed as the RVF virus is used and thus extra precautions are needed to be in place when conducting these experiments. Confirmation of clinical cases is made through RT-PCR, cell culture, antigen detection (e.g. IgM ELISA) or histopathology. However viremia is short-lived and PCR readings start to decline by day 4 of infection (Weingartl et al. 2014, Stedman et al. 2019)

1.5. Structure of RVFV and virus life cycle

In common with other bunyaviruses, RVFV has a tripartite genome that consists of three single-stranded RVF segments (large, medium and small). The large (L) and medium (M) segments are negative sense, like most viruses in the Bunyavirales order the S segment is ambisense. This is specific to the Phlebovirus family (Figure 1.1) (Ikegami et al. 2011, Brennan et al. 2014). The RVF virions are spherical and are approximately 80-120nm in diameter. RVFV has a lipid envelope made up of two glycoproteins Gn and Gc which are encoded by the M segment. These glycoproteins form heterodimers which form the icosahedral symmetry of the virus envelope and aid in cell entry. The M segment also encodes two non-structural protein NSm1 (78kDa) and NSm2 (14kDa). The NSm is important for RVFV infection and transmission in *Aedes* mosquitoes (Crabtree et al 2012). The L segment encodes the viral RNA dependent RNA polymerase and is responsible for replication and transcription along with the ribonucleoprotein (RNP) (Lopez et al. 1995). The S segments is ambi-sense, it encodes the NSs non-structural protein in the positive sense and nucleoproteins which make up the nucleocapsid (N) in the negative sense. Nucleoproteins regulate genome packaging, encapsidating segments individually within the virion. The N protein also protects the RNA from degradation in the form of RNP complexes and is essential for transcription of RNA (negative sense genomes are unable to transcribe non-encapsidated RNA) (Raymond et al 2010). The N protein is also important in viral assembly as it helps produce virus-like-particles (Piper et al. 2011). For each segment, the terminal eight nucleotides are highly conserved across *Phleboviruses* (Ikegami et al. 2012).

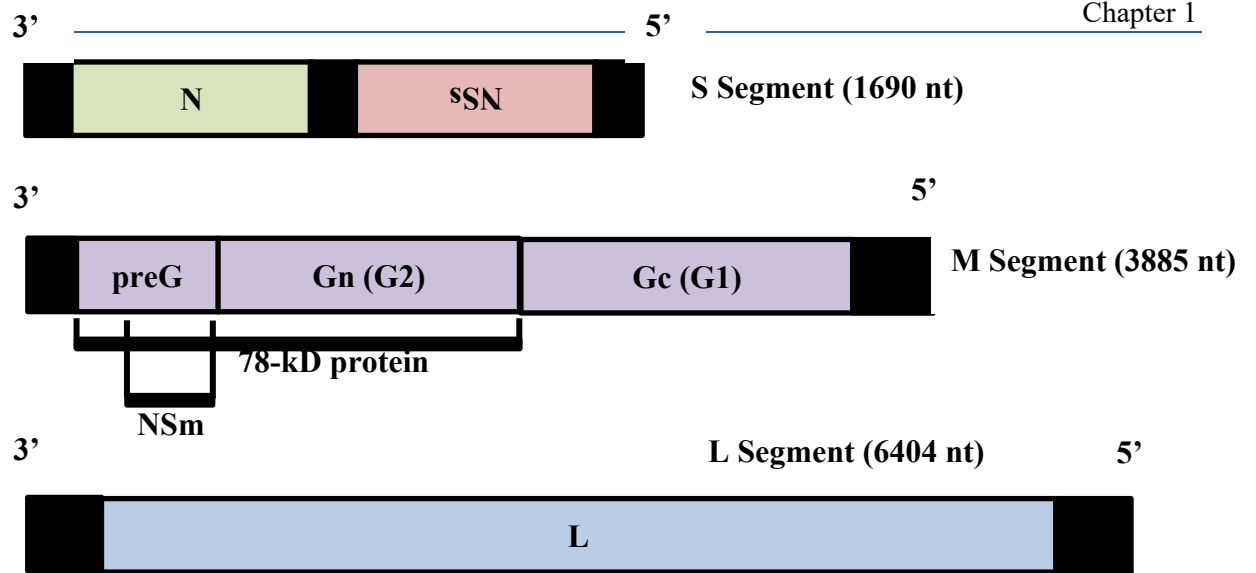


Figure 1.1 RVFV genome structure

Adapted from Ikegami and Makino (2011). The S segment encodes the N and NSs in an ambi-sense manner, they are encoded in opposite orientations.

A study has shown that RVFV (MP-12 strain) enters mammalian host cells via caveola-mediated endocytosis (Harmon et al. 2012). Many viruses enter cells using this mechanism. Caveola are scaffolding proteins which are found on the plasma membrane, they produce signals to mediate entry of the virus into the cell (Harmon et al. 2012). Once within cells the ribonucleocapsid is released into the cytoplasm. Primary transcription occurs very quickly, transcribing the N protein and the NSs protein and RNA replication occurs around 1-2 hours post-infection. The NSs protein is a major virulence factor for RVFV (Vialat et al. 2000).

1.6. Phylogeny

Bayesian analysis (time to most common recent ancestor, TMRCA) was conducted on 33 RVF genomes has indicated that RVF genomes have diverged from an unknown arbovirus during the 1880-1890s (Bird et al. 2007). To date, only one serotype has been identified, but there are a number of strains that are classified into genetic lineages. Isolates have been sequenced into 15 lineages based on a 490-nt section of the Gn glycoprotein. Some lineages do cluster in the same geographical regions (Grobbelaar et al. 2011). Circulating RVF virus strains have shown limited genetic diversity (5% at nucleotide level and 2% at amino acid level) (Bird et al. 2007).

There is broad circulation of these lineages across East Africa, however many have originated from Kenya (Samy et al. 2017). Different strains can cause different severity of disease (Wilson et al. 2016). It has been postulated that RVFV, as well as other arboviruses, have limited diversity due to the fact they alternately replicate in both mammals and arthropod hosts. Moutailler et al. (2011) illustrated that alternating replication is necessary to maintain the NSs virulence factor. Evolutionary dynamics of RVFV also suggests that

recombination occurs through reassortment but at a very low rate and that the three segments have distinct evolutionary dynamics (Bird et al. 2007, Freire et al 2015).

1.7. Immunology

A correlate of protection is defined as a mechanism or immune marker that causes protection by interrupting the pathway between exposure or infection (World Health Organisation, 2013). It has previously been shown that passive transfer of neutralising antibodies against the glycoproteins of RVFV, Gn and Gc, are sufficient for protection from lethal RVF and are considered a correlate of protection when titres reach those seen from naturally infected animals (Schmaljohn et al. 1989, Smithburn et al. 1949). Antibodies to the nucleoprotein, and the non-structural protein are also produced at a lower level but are not associated with protection (Pepin et al. 2010). Within a week of being infected RVF-specific IgM antibodies are produced but these drop after 50 days (Niklasson et al. 1984, Paweska et al. 2003). RVF-specific IgG antibodies appear within 3 weeks after the IgM response. Not all IgG antibodies will be neutralising but those that are provide cross-strain immunity and are long lived (Sabin and Blumberg, 1947, Brown et al. 1957, Bird and Nichol, 2012).

Little research has assessed the cellular response to RVF infection. One study assessed the peripheral immune response during infection of lethal or sublethal RVF in African Green Monkeys (AGMs). Neutralising antibody responses developed at similar levels in both groups however there was a distinct difference in T cell response. Total numbers of CD4⁺ and CD8⁺ T cells increased causing more expression of MHC class II molecules in AGMs that survived compared to those that succumbed to infection (Wonderlich et al. 2018). Another paper also found that the Gn and Gc glycoproteins carry immunodominant CD8⁺ epitopes in mice (Warimwe et al. 2013).

1.8. Transmission cycle and viral maintenance

The transmission cycle of RVFV typically has two phases: (1) the enzootic inter-epidemic period (IEP) where low level circulation occurs, (2) Epizootics following heavy, persistent rainfall causing flooding events.

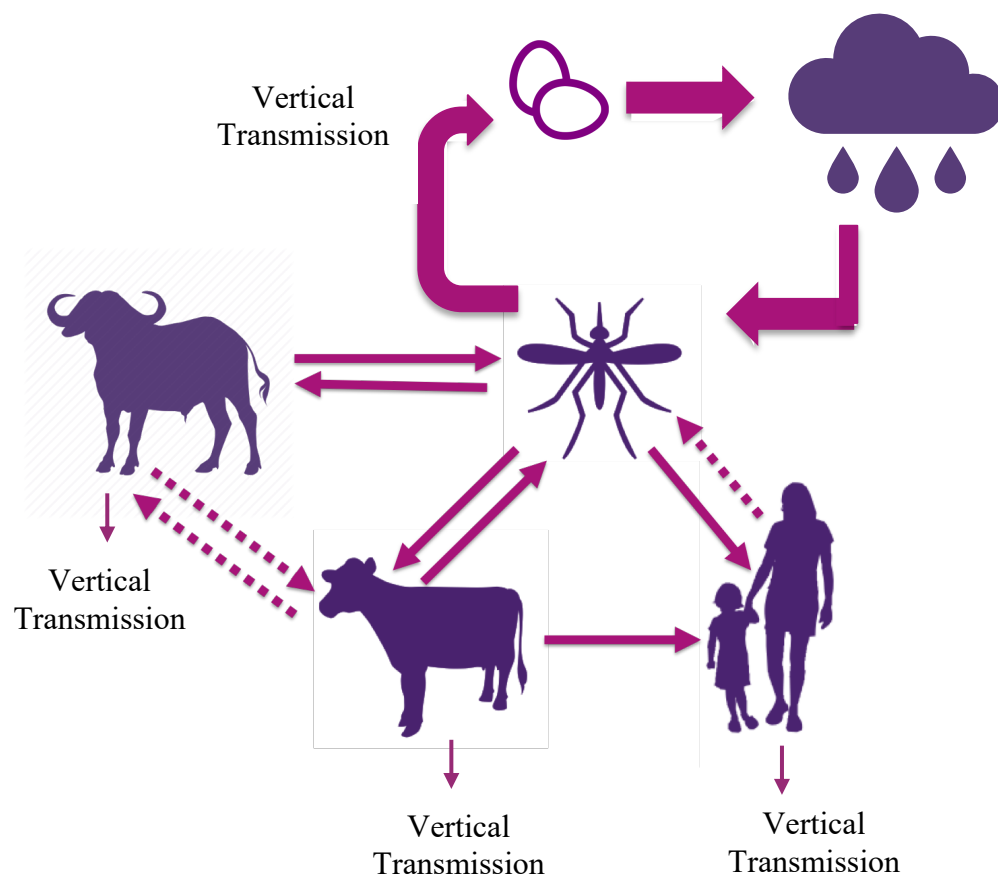


Figure 1.2 RVF Transmission Cycle

Intervals when outbreaks are not occurring are known as interepidemic or interepizootic periods (IEPs). Maintenance of the RVF virus during these IEPs is not completely understood but there are a number of established theories. During IEPs it has been hypothesised that infected *Aedes* mosquitoes are able to transmit the virus to their offspring via transovarial transmission (Linthicum et al. 1985) (Figure 1.2). Although direct evidence of this has only been recorded in a limited number of papers and only in one *Aedes* species (Linthicum et al. 1985, Romoser et al. 2011), misidentified as *Aedes lineatopennis*, now identified as *Aedes (Neomelaniconion) mcintoshi* (Huang, 1985), it is accepted that *Aedes* spp. are able to transmit transovarially (Li et al. 2017, Bergren et al. 2018). *Aedes* spp. can also lay eggs without a blood meal (autogeny). This is dependent on environmental conditions including high temperature, good larval nutrition and sugar feeding. Although unlikely to sustain populations this may provide support in maintaining the virus during IEPs and when there are no hosts around to blood feed (Ariani et al. 2015). *Aedes* mosquito eggs, including those infected through transmission are able to survive hostile environments. *Aedes* eggs are resistant to drought and are able to survive on dry soil for as long as 15 years. Often eggs are found in areas which have shallow, depressed habitats known as dambos.

No mammalian maintenance hosts have been recorded for RVFV although seroconversion has been identified in multiple wildlife species including African buffalo, elephant, warthog, black rhino, common zebra, Thomson's gazelle, lesser kudu, impala and waterbuck (Evans et al. 2008). Clinical signs of infection have been recorded in African buffalo where one animal aborted (Davies and Karstad, 1981) and virus has been detected in springbok (Capobianco Dondona et al. 2016). During IEPs, low-level circulation in wildlife and livestock may occur (Linthicum et al. 2016), suggesting the RVFV lifecycle is not dependent on *Aedes* alone (Rostal et al. 2017, Manore and Beechler 2015). It is unknown whether wildlife species act as RVFV reservoirs, though it has been suggested that it may not be one species alone but a reservoir system (Haydon et al. 2002).

When heavy, persistent rainfall occurs these infected *Aedes* eggs emerge with high viremia and feed on livestock species causing onward transmission (Davies et al. 1985) (Figure 1.2). Other mosquito species (such as *Culex spp.* or *Anopheles spp.*) may become infected when feeding from infected livestock acting as secondary vectors amplifying transmission and causing an epizootic. Outbreaks in East Africa typically rely on unusually heavy rainfall to occur as well as susceptible populations being present. As concerns increase with the potential of climate change, the impact RVFV has may expand to new regions (Lo Iacono et al. 2018).

Routine surveillance for RVFV in African countries is limited and outbreaks are underreported (Davies et al. 2010). Proxy measures such as the normalized difference vegetation index (NDVI), monitoring of the El Niño Southern Oscillation (ENSO) events and the sea surface temperature (SST) anomalies between Indian and Atlantic oceans have been used to predict when and where RVF outbreaks may occur (FAO, 2013, Anyamba et al. 2009, Redding et al. 2017).

1.9. Geographical distribution of the virus

RVF was first reported in a flock of sheep in Naivasha in the Rift Valley of Kenya in 1930 (Daubney et al. 1931), however reports of enzootic hepatitis in sheep were observed in 1912 (Stordy, 1913). Since then many lineages of RVFV have originated from the country based on low genetic diversity which separated different isolates into lineages but are not different enough to be different serotypes (Grobbelaar et al. 2011, Samy et al. 2017). Over the last 90 years there have been multiple outbreaks across the African continent as well as in Saudi Arabia and islands in the Indian Ocean (Naninygi et al. 2015). A study has found RVFV seropositive camels, gazelles and water buffalo in Turkey (Gür et al. 2017), supporting

concerns that there may be RVFV in Europe, as has been seen with other vector-borne diseases such as Bluetongue virus (BTV), West Nile virus (WNV), Chiungunya virus and Zika virus.

Outbreaks of the disease have been reported in many African countries (Nanyingi et al. 2015) with outbreaks in livestock species typically proceed those in humans. Major epidemics have included: 1950-1951 and 2009 in South Africa; 1977 in Egypt; 1997-1998 in Kenya and Somalia; 2006-2008 in Somalia, Kenya, Sudan, Madagascar and Tanzania; 2010-2011 in South Africa and 2019 in Mayotte. In between these large outbreaks, an increase in reporting of pockets of outbreaks in various African countries has also been recorded. The outbreak in South Africa in 2009 was notable as it occurred in the absence of heavy rainfall and it caused high mortality in pregnant cattle (Maluleke et al. 2019). The Arabian Peninsula outbreak in 2000 was the first to take place outside of the African continent. This outbreak had a huge impact on livestock through abortions and mortalities, affecting approximately 40,000 animals (Al-Alfaleq et al. 2011) and 886 human cases were reported, with a key manifestation being hepatitis (Madani et al. 2003). Kenya has reported the highest number of RVF outbreaks making it a suitable target country for implementing control measures.

1.10. RVF and Kenya

As reported in the previous section (1.9.) Kenya was the first country to formally report RVF. Since the 1912 and 1930 outbreaks there have been multiple outbreaks reported in 1968, 1978-1979, 1997-1998, 2006-2007 (Nanyingi et al. 2015). Additional seroprevalence studies have also reported seropositive animals and humans in certain regions of the country (Labeaud et al. 2007, Labeaud et al. 2015, Lichoti et al. 2014).

Agriculture is a huge component of the Kenyan economy contributing 26% of the Gross Domestic Product (GDP) and employing more than 40% of the total population (Total population 2019: 52.5 million) (FAO, 2017, World Bank, 2020). In 2015 the total population of cattle was estimated at 18 million, sheep 18 million, goats 28 million and camels 3 million with approximately 7 million (60%) of households keeping livestock (FAO, 2017). RVF outbreaks can have direct and indirect impact on not just livestock management and welfare but also on public health, livelihoods and environmental threats (FAO, 2017). Therefore, control strategies or preparedness for outbreaks are needed.

Although Kenya has a diverse environment, a large majority of the country has optimal conditions for RVF maintenance, and, hence potential outbreaks. The 2006/2007 RVF outbreak in Kenya caused losses of over \$32 million to the national economy (Gachohi et al. 2016), 120 deaths and 411 reported cases in humans and the deaths of over 900,000 heads of livestock (cattle, sheep, goats and camels) (FAO, 2017). This outbreak caused a huge impact through animal deaths, market closures, illness and limited sales of meat and animal products, and it also has a large impact on other stake holders who are part of the livestock value chain (Rich et al. 2010).

Although the Kenyan vaccine manufacturer KEVIVAPI manufactures the Smithburn vaccine, uptake of vaccination against RVF is low in Kenya: in 2007 only 11% of cattle, 1.2% of camels, 4.8% of sheep and 5.9% goats were vaccinated (Kimani et al. 2016) and outbreaks continue to be reported (OIE 2019).

1.11. Control of RVF

As the virus is a vector-borne, multi-host pathogen that can stay dormant in *Aedes* eggs for multiple years before emerging the strategies to control the disease are complex and require a One Health response (Fawzy et al. 2019).

Mosquito control is not the optimum control strategy for RVF. It is expensive, it can be impractical and can have negative effects on the environment. Vector control, particularly larvicidal treatment, may work if targeted at well-defined breeding and resting sites (FAO, 2002). Public awareness programmes typically discussing the dangers of contact with infected animals and slaughtering processes are particularly important for controlling spread in humans as well as promoting the use of personal protection such as the use of mesh screens on windows and bed nets (Alam and Mohammed, 2000, Davies 2010).

Restriction of movement particularly for trade purposes and across country boundaries can also be deemed a control measure although this would not be enough alone and issues with illegal trade could still cause onward transmission (Napp et al. 2018, Métras et al. 2017). Quarantine and culling of animals are a difficult measure to implement due to the zoonotic nature of the disease and also being able to have sufficient tests that can differentiate between infected and vaccinated animals.

Having regular serological and risk-based surveillance is a powerful preparedness strategy in terms of RVF control prior to outbreaks occurring. Surveillance systems could include sentinel herds which are monitored over multiple years as well as climatic and risk-based monitoring which would provide useful information for targeting control strategies. The optimal control measure at present is livestock vaccination which also acts as a control measure for transmission into humans as well. RVF livestock vaccination is therefore a major topic of this thesis.

1.12. What is expected from a RVF vaccine?

The OIE Terrestrial manual (OIE, 2018) summarises desired characteristics for a RVF vaccine which the pharmaceutical industry coin as a Target Product Profile (Lee and Burke 2010):

“1.5 Endemic regions

The objective is the prevention and control of epizootics and epidemics in endemic areas and to contribute to the improvement of livestock production in endemic areas. In order of priorities, characteristics of the vaccines are:

1. preferably one dose, resulting in a long-lasting immunity of at least 1 year;
2. preferably a life-long immunity after a limited number of doses.

1.6 Free or non-endemic region

Vaccines would be used either for the prevention of, or the response to an introduction of the virus. The expected characteristics of the vaccines are: safe with a quick onset of protective immunity and protection in animals of all ages and physiological status. Although DIVA (detection of infection in vaccinated animals) is an important property of any future vaccine, a requirement for DIVA should not hinder or block the development or licensing of an effective RVF vaccine.

In all cases, the vaccines should be:

1. Safe for the staff involved in the production of the vaccines and for the users, safe to all physiological stages of animals, and with minimal risk of introduction into the environment (potential vectors);
2. Protective in multispecies and if possible in all susceptible species of economic importance, to prevent infection and transmission;

3. Cost effective for producers and users, preferentially with a single-dose vaccination;
4. Easy to use (e.g. needle-free delivery), suitable for stockpiling (e.g. a vaccine bank) and quick availability. ”

Extracted from The OIE Terrestrial Manual (OIE, 2018)

Minimal risk of introduction to the environment is also a consideration, specifically in terms of viral vector vaccines.

Targeting livestock species for vaccination against a zoonotic pathogen is an example of the role veterinary vaccines can play in public health (Roth, 2011). This has more recently been reported for a vaccine for Middle East respiratory syndrome coronavirus (MERS-CoV) for camels (Alharbi et al. 2019).

1.13. Veterinary Vaccines for RVFV

At the present time there are four veterinary vaccines that have been commercialised for RVFV (Smithburn, Clone 13, and two inactivated vaccines: binary ethylenimine-inactivated RVF ZH501 vaccine, formalin-inactivated RVF-Menya strain-based vaccine), although they do not meet the desired requirements outlined by the OIE. The most commonly used is the Smithburn vaccine: a live attenuated vaccine that was developed in 1944 from a neutrotropic RVFV strain isolated in Entebbe from a mosquito *Eretmapodites* spp. (Smithburn 1948). The vaccine was serially passaged by intracerebral inoculation in mice and later a neuroadapted strain was established during serial passages, which led to the creation of the master seed virus used for Smithburn vaccines (Smithburn 1949a). The duration of protective immunity in ruminants and cattle is debatable with some studies suggesting long-term protective immunity is provided with a single dose (Coackley and Gosden 1967) and

others suggesting immunity wanes significantly within a year. This depends on vaccine batch, ELISA sensitivity and species type (Ahmed et al. 2007).

Due to being a live RVFV, the Smithburn vaccine should not be used in outbreak settings due to the possibility of genetic reassortment with natural pathogenic RVF isolates or reversion to virulence which could cause further outbreaks. This is particularly important when an outbreak is ongoing and a vaccine is administered to an animal which is already infected: these vaccines often come as multidose vials and needles are not necessarily changed between animals (Davies et al. 2010). Smithburn vaccine is contraindicated in pregnant animals as it can cause abortions similar to wild type RVFV (Botros et al. 2006). Diagnostic tests are unable to differentiate between infected and vaccinated animals (DIVA).

The inactivated vaccines elicit neutralising antibodies in vaccinated livestock, but frequent booster vaccinations are needed (Faburay et al. 2017). This can be an issue for resource-limited settings and for transhumance and pastoral communities who would be target populations for such vaccines (Barnard et al. 1979, Dungu et al. 2013, Faburay et al. 2017).

The MP-12 vaccine is another live attenuated vaccine developed from the ZH-548 strain under development which was isolated from a human patient. It has yet to be commercialised. Following twelve passages in the presence of 5-fluorouracil, it was found to be attenuated in hamsters (Saluzzo et al. 1990). The vaccine has been tested in both livestock and humans (Peters 1997, Faburay et al. 2017). Neutralising antibodies were induced in ewes vaccinated at 70-100 days of gestation and vaccination did not result in any abortions or abnormalities (Morrill et al. 1991, Baskerville et al. 1992). However, abortions were recorded in 4% of ewes and teratogenic effects were recorded in 14% of newborn lambs causing hydranencephaly, microencephaly and arthrogryposis in another study in which ewes were vaccinated at day 28 of gestation (Hunter et al. 2002).

Significant concerns with previous vaccines developed against RVF, including the need for multiple doses and the risk of abortion and fetal malformation in pregnant animals, meant alternative vaccines were still needed. Clone 13 is a plaque-derived clone of RVFV isolated from a human patient from the Central African Republic during the 1974 outbreak (74HB59 strain) (Muller et al. 1995). The vaccine was attenuated in mice and had a natural deletion in the NSs gene which is responsible for viral virulence. Although studies showed it was protective and did not cause abortion or teratogeny in pregnant ewes (Dungu et al. 2010), the vaccine can cross the placental barrier causing infection, malformations and stillbirths when vaccinated with an overdose in ewes in their first trimester (Makoschey et al. 2016). This vaccine has been registered in South Africa, Botswana and Namibia. It has successfully been developed to be thermostable (Clone 13T vaccine) and this vaccine has been registered in Morocco (Daouam, et al. 2014, Dungu et al. 2018). This vaccine is also DIVA compatible and seroconversion is persistent for a year after vaccination in sheep and goats (Lo et al. 2015). There are still concerns regarding the possibility of genetic reassortant with the Clone 13 S segment and pathogenic field strains.

Other vaccines including recombinant protein vaccines, virus like particle (VLP) vaccines and DNA vaccines are also under development for use as RVF vaccines (Faburay et al. 2017).

1.14. ChAds and ChAdOx1 RVF

Viral vectors, specifically adenoviruses are an established platform for rational vaccine design for both clinical and veterinary use. They are able to produce humoral and cellular mediated antigen-specific responses (Ewer et al. 2013, Ogwang et al. 2015, Warimwe et al. 2016, Ewer et al. 2016), can be administered via injection or orally (Zhou et al. 2006) and can grow to high titres (Ewer et al. 2017). Furthermore, they have the capacity to insert genes

of interest (vaccine specific-antigens) up to 8kb in size (Dicks et al. 2012) and are thermostable (Dulal et al. 2016): all properties important for deploying vaccines and maintaining a cold chain in target resource-limited countries. By only incorporating a small part of the virus into the vaccine, diagnostic tests utilising a different part of the virus should be able to differentiate between infected and vaccinated animals when these vaccines are used which is important during outbreak situations. Replication-deficient adenoviruses are engineered to have the E1 gene deleted which is needed for viral replication. This is also where the foreign genes of the target pathogen for the vaccine are inserted (Dicks et al. 2012).

The chimpanzee adenovirus has recently been developed as a viral vector to circumvent issues with pre-existing immunity to human adenoviruses (Morris et al. 2016). A ChAd virus was therefore chosen to be used as the viral vector for the RVF vaccine as the larger programme of work will include making a vaccine for human use. The ChAdOx1 viral vector was developed by the Jenner Institute, University of Oxford. It is derived from the simian (*Pan troglodytes*) adenovirus Y25 (Dicks et al., 2012). The Y25 virus is grouped in human adenovirus E species. To use the virus as a viral vector it was incorporated into a bacterial chromosome system (BAC) which produces clones that can be genetically modified through BAC recombineering rather than using plasmid (Dicks et al., 2012). The removal of the E1 and E3 genes of the Y25 virus (as well as other simian adenoviruses) renders it replication deficient and provides the large insert capacity for incorporating the target vaccine antigen.. ChAdOx1 has already been used as a vaccine vector for malaria (NCT03203421), chikungunya (NCT03590392), influenza (NCT01623518) and TB (NCT018294490), amongst other diseases. It has also been tested in dromedary camels as a vaccine for Middle East respiratory syndrome coronavirus (MERS) (Alharbi et al. 2019).

ChAdOx1 is a chimpanzee adenovirus that has been adapted for use as a viral vector for RVFV, ChAdOx1 RVF. Neutralising antibody responses to the ChAdOx1 RVF vaccine have been reported in mice and multiple livestock species (Warimwe et al. 2013, Warimwe et al. 2016 and Stedman et al. 2019). Further to this, it has a well-established efficacy and safety profile in livestock species including sheep, goats, cattle and camels (Warimwe et al., 2013, Warimwe et al., 2016, Stedman et al., 2019). Sheep, goats and cattle were vaccinated with ChAdOx1 RVF, ChAdOx1 RVF and adjuvant or a Phosphate Buffer Saline (PBS) control group and challenged with a virulent RVFV strain in high containment facilities in Kenya. All animals vaccinated with the ChAdOx1 RVF or ChAdOx1 RVF and adjuvant elicited high levels of neutralisation antibodies, showed no adverse effects and, no viraemia was reported in the ChAdOx1 RVF vaccinated animals (Warimwe et al., 2016). The results from the ChAdOx1 RVF and adjuvant group showed there was no need for an adjuvant, the vaccine worked just as well without one (Warimwe et al. 2016). In this same paper, dromedary camels were also vaccinated, and immunogenicity testing was carried out (there was no challenge). The vaccinated camels also produced neutralising antibodies. This study was conducted as a proof of concept, efficacy and safety study, with a small sample size of animals.

Following on from the multispecies efficacy study a vaccine efficacy and safety study was conducted in pregnant sheep and goats in their second trimester (Stedman et al. 2019). The vaccine was safe and protected against viraemia and foetal loss in sheep and goats. Results suggested that the vaccine elicits sterile immunity in sheep however this was not the case for goats as viral RNA was detected in both maternal and foetal tissue (Stedman et al. 2019). Despite the high titre neutralising antibodies in pregnant goats, it was reported that half of the does had evidence of viral RNA in plasma or placentomes and in one of the placentomes live virus was isolated. This may have occurred due to host differences in immune mechanisms needed for protection in utero infection in goats (Stedman et al. 2019).

Similar to natural immunity, protection is elicited through a strong neutralisation antibody response to the Gn and Gc glycoproteins of RVFV. It is currently unknown what neutralising antibody titre threshold is needed for protection against the virus. It has therefore been assumed that animals would be to be protected by a vaccine if their antibody titres are in the range generally following natural infection albeit a much lower titre than natural response may be sufficient. Preliminary studies in mice showed that T cells may play a role in protection as well and CD4⁺ T cells can influence immunity against RVF neuropathology in mice. However, much is still unknown about roles T cell phenotypes may have in immune protection against RVFV (Warimwe et al. 2013).

Unlike the Smithburn vaccine, the ChAdOx1 RVF vaccine encodes the glycoproteins of the virus only, circumventing the contraindication of use during outbreaks. As only the glycoproteins are encoded the risk of genetic reassortment with wildtype virus is considerably lower than with live attenuated vaccines such as the Smithburn vaccine or even Clone 13. For DIVA compatibility, the most widely used commercial ELISA kit uses the RVF nucleoprotein as the target antigen, as the ChAdOx1 RVF does not encode the nucleoprotein any positives recorded on the commercial test should be infected animals alone.

The ChAdOx1 RVF vaccine is being co-developed for human and susceptible animal species. It will be the first 'One Health' vaccine and will pave the way for future control of emerging pathogens that are capable of zoonotic transmission (Warimwe et al., 2016). ChAd vaccine development requires cell-based production and downstream purification phases which differ for human and veterinary vaccine production.

Maintaining a cold chain for vaccines is a major hurdle in vaccine deployment, particularly in settings where there may be issues with interrupted electricity and poor transport infrastructure. In this case having a thermostable vaccine is a huge advantage. A thermostable ChAdOx1 RVF vaccine kept at 25, 37 and 45°C for 6 months is able to elicit comparable neutralising antibody responses to ChAdOx1 RVF stored at -80°C that protect against infection (Dulal et al., 2016).

1.15. Mathematical modelling of Rift Valley fever transmission dynamics

Mathematical models are a powerful tool in predicting and understanding transmission dynamics and control measures such as vaccination for sporadic outbreak infectious diseases such as RVFV. They are a conceptual tool which allow the user to predict and gain insight into how a system will behave, particularly as infectious disease (unlike chronic conditions) depends on the state of other individuals in a population as well as personal risk factors (Lloyd-Smith et al. 2009).

There are still many unknowns regarding the interepidemic nature of RVF maintenance in the environment, the ability for the virus to emerge, definitive correlates of protection and inter- and intra-species transmission to name a few. As Keeling and Rohani (2008) emphasise there is a trade-off between accuracy to reproduce and predict dynamics which relies on the understanding and availability of disease history and relevant parameters; transparency in understanding how various components interact and influence disease dynamics achieved by building on simple established models; and, finally, flexibility, allowing the model to be adaptable for various situations.

Reviews have outlined contributions to zoonotic disease and specifically the RVF modelling field, stressing the complexity of multi-host ecology of such zoonotic infections (Lloyd-Smith et al. 2009, Métras et al. 2011, Danzetta et al. 2016). Models assessing impact of vaccination have successfully been generated and used to guide policy for many veterinary and zoonotic diseases including blue tongue virus (Szmaragd et al. 2010), foot-and-mouth disease (Keeling et al. 2003), swine influenza (Reynolds et al. 2014) and rabies (Zinsstag et al. 2017).

Transmission models for infectious diseases are best described through compartmental models which reflect the natural history of the disease. One of the simplest of these is compartmentalising populations into those susceptible (S), infected (I) and recovered (R) groups with regard to disease status (Vynnycky and White, 2010). The method of modelling is determined as deterministic: what happens on average to a population or stochastic: which allows there to be an element of chance with regard to individuals moving between compartments. The latter model method will therefore provide a range of how often the outcome (e.g. number of cases) will occur and is particularly helpful for making policy decision such as vaccination strategies.

These types of models are therefore used for predicting emergence (Fischer et al. 2013, Métras et al. 2017) and understanding how control strategies can mitigate these outbreaks. Previous models have compared different types of RVF vaccines (Live vs. Killed) (Chamchod et al. 2015) whilst others have assessed the best way of implementing a vaccination campaign to reduce cases during outbreaks (Gachohi et al. 2016).

1.16. Research Project and thesis

This thesis links field epidemiology, laboratory work and mathematical modelling forming an integrated investigation into the role of veterinary vaccination in the control of Rift Valley fever in Kenya.

Chapter 2: Seroprevalence of Rift Valley fever virus in Africa: a systematic review

The aim of this chapter is to critically evaluate RVFV seroprevalence in livestock, wildlife and humans across the African continent, as well as identifying risk factors for seropositivity.

Objectives

1. Design and follow a protocol for searching and analysing suitable papers on RVF seroprevalence in livestock, wildlife and humans following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.
2. Analyse spatio-temporal distribution of RVF seroprevalence
3. Assess risk factors associated with RVF seroprevalence
4. Evaluate bias in study design and data analysis methods

Chapter 3: Seroprevalence of Rift Valley fever virus in Kenya

This chapter investigates RVF seroprevalence in livestock in different counties during an interepidemic period in Kenya.

Objectives

1. Utilising samples from the Population-Based surveillance system in Kisumu County, test livestock serum samples from 2013-2016 for RVF antibodies.

2. Sample livestock in Laikipia and Machakos county in 2018 and 2019 and test samples for RVF seropositivity.
3. Analyse livestock samples to elucidate if there is any demographic significance in RVF seropositivity.

Chapter 4: Chimpanzee Adenovirus Viral Vector Immunogenicity in the context of use as a Rift Valley fever vaccine

The aim of this chapter is to investigate immunity to the chimpanzee adenovirus used in the ChAdOx1 RVF vaccine in multiple livestock species.

Objectives

1. Assess pre-existing immunity to ChAdOx1 in livestock species from Kenya, UK and Saudi Arabia.
2. Compare anti-ChAdOx1 immunity before and after vaccination in multiple livestock species
3. Evaluate anti-vector immunity in sheep and cattle vaccinated in a vaccine dose escalation study.
4. Elucidate if anti-ChAdOx1 immunity correlates with RVF antigen immunity and hence vaccine efficacy.

Chapter 5: A non-inferiority Rift Valley fever vaccine field trial to assess vaccine immunogenicity in multiple livestock species in Kenya: Study design and regulatory perspective

To design and implement a randomised, blinded, veterinary Good Clinical Practice (vGCP) non-inferiority field trial comparing the immunogenicity and safety of a novel RVF vaccine with commercialised Riftvax in multiple livestock species.

Objectives

1. To develop a field trial protocol assessing immunogenicity and safety of the ChAdOx1 RVF in multiple livestock species meeting the requirement of vGCP.
2. To liaise with government officials and external monitoring teams to achieve approval to commence the vaccine field trial.
3. To evaluate how to implement a vGCP study in a large field trial in an endemic setting

Chapter 6: A non-inferiority Rift Valley fever vaccine field trial to assess vaccine immunogenicity in multiple livestock species in Kenya: Vaccine safety

To evaluate the safety of ChAdOx1 RVF in comparison with Smithburn vaccine and placebo control in four livestock species.

Objectives

1. Record critical safety observations, injection site reaction observations and rectal temperatures under the principles of vGCP on specified days of the vaccine field trial
2. Describe results of clinical observations, reporting type of observation, proportion of animals in the different vaccination groups to have adverse events, duration of these reactions and if there was any significance by treatment group
3. Report any field trial mortalities and post-mortem diagnosis and if these were attributed to the vaccine administered.
4. Evaluate injection site reactions by proportion of animals in the different vaccination groups as well as duration of reactions and if there was any significant by treatment group
5. Report rectal temperatures by species and treatment group

Chapter 7: A non-inferiority Rift Valley fever vaccine field trial to assess vaccine immunogenicity in multiple livestock species in Kenya: Vaccine immunogenicity

To measure vaccine immunogenicity analysis in multiple species to assess non-inferiority of the ChAdOx1 RVF as well as assess its DIVA capabilities.

Objectives

1. Collect blood samples from all animals recruited into the non-inferiority vaccine field trial at day -1 pre-vaccination and day 28 post-vaccination.
2. Conduct laboratory analysis of these samples on a commercial competitive ELISA to assess DIVA capabilities
3. Analyse results from the DIVA ELISA and RVF FRNT in relation to vaccine allocation
4. Determine whether the ChAdOx1 RVF vaccine is non-inferior to the Riftvax vaccine by conducting non-inferiority statistical analysis

Chapter 8: Modelling the effect of vaccination on RVFV transmission dynamics

To develop a stochastic multi-host transmission dynamics model for Rift Valley fever virus and use this to assess the impact of livestock vaccination as a suitable control measure in Kenya.

Objectives

1. Using transmission data from published literature and seroconversion data from the vaccine field trial, develop a stochastic multi-host and vector transmission model.
2. Model RVFV transmission dynamics over a 25-year period in two livestock species and one mosquito vector species

3. Model the impact vaccination has on RVF transmission and hence cumulative incidence of infected livestock species and vectors considering vaccine efficacy, frequency of vaccination and pre-emptive vs. reactive vaccination.

Chapter 9: Discussion

The discussion concludes the thesis by addressing the significance of the thesis findings, providing merits and limitations to the approaches used within the context of wider research and application.

2. Seroprevalence of Rift Valley fever virus in Africa: a systematic review

Elements of this chapter have been published in the following peer-reviewed article:

Clark, M. H. A., Warimwe, G. M., Di Nardo, A., Lyons, N. A. & Gubbins, S. Systematic literature review of Rift Valley fever virus seroprevalence in livestock, wildlife and humans in Africa from 1968 to 2016. *PLoS Negl. Trop. Dis.* **12**, e0006627 (2018).

2.1. Background

This chapter seeks to evaluate RVFV seroprevalence across the African continent in livestock, wildlife and humans in order to understand the spatio-temporal distribution of RVFV seroprevalence and to identify knowledge gaps and areas requiring further research. Risk factors associated with seropositivity were identified and study designs evaluated to understand the validity of their results.

Assessing historic and present RVFV seroprevalence in livestock, wildlife and humans within African countries provides evidence of where the virus may circulate and helps identify at-risk populations, potentially informing intervention strategies and resources allocation. This is important because: (i) there is a global concern that RVFV is a pathogen that has the potential to cause large-scale epidemics (World Health Organisation, 2015); (ii) in 2000 the geographical range of RVFV epidemics extended to the Arabian peninsula (Madani et al. 2003) and (iii) the theoretical ease with which infection could be sustained in other parts of the world due to favourable ecological conditions, including the presence of competent vectors (Rolin et al. 2013, Lorenzo et al. 2015, Fischer et al. 2013). By implementing control measures in at-risk populations during inter-epidemic years, further dissemination of the disease can be prevented.

A systematic review was conducted for this piece of work. Systematic reviews are a structured literature review that uses a pre-defined methodology developed by the user but following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) checklist to collect and critically evaluate secondary data (Moher et al. 2009, Moher et al. 2015). The PRISMA checklist provides a checklist for necessary components for conducting a systematic review (Appendix A2.1). The pre-defined methodology forms a protocol which is determined prior to conducting the review. This enables the review to be

conducted minimising bias as well as providing transparency and research integrity. This produces a complete summary of all data available to answer the research question.

This chapter seeks to assess RVFV seroprevalence in wildlife, livestock and humans on the African continent, particularly considering the relationship between these species and the risk of RVFV spillover transmission. In order to do this comprehensively, the study designs of eligible articles were evaluated to examine how seroprevalence was measured and calculated, including potential sources of bias. Reviews of Rift Valley fever epidemiology have evaluated the spatio-temporal (Clements et al. 2007), ecological, predictive risk factors (Nanyingi et al. 2015) and modelling methods (Métras et al. 2011) used in studies across Africa. Previous seroprevalence studies in wildlife, livestock or humans have typically focused on a single time point, species and country. This review will bridge these studies, identify associated trends in RVFV seroprevalence and evaluate study designs.

2.2. Methods

The study protocol for this systematic review used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al. 2009) (Appendix A2.1). A search of published studies on MEDLINE (PubMed) and Web of Knowledge was carried out on 8th January 2018; all articles that could be accessed on these databases were considered for inclusion in the review. Search terms used to identify articles were: ((“Rift Valley Fever” OR “RVF”) AND (“prevalence” OR “incidence” OR “sero*”). Broad search terms were purposefully used to maximise the chances of capturing all relevant research articles. Data extraction, screening and analysis were carried out by myself, with co-authors providing advice when required. An Excel spreadsheet was used to record data from eligible studies. Data extracted included: (i) seroprevalence percentage (by species, year of study and country); (ii) sample size; (iii) type of study; (iv) diagnostic and statistical

tests used and (v) information on study design. The excel spreadsheet can be found in the supplementary information of the published systematic review (Clark et al. 2018).

For a research article to be included in the review it had to meet the following criteria: (i) focused on an African country; (ii) provide original quantitative serological information on RVFV in humans, livestock and/or wildlife; and (iii) be available in the English language. Studies were excluded if: they had utilised previously published seroprevalence datasets for further analysis (including mathematical modelling), or they had not recorded the year of sampling (Figure 2.1).

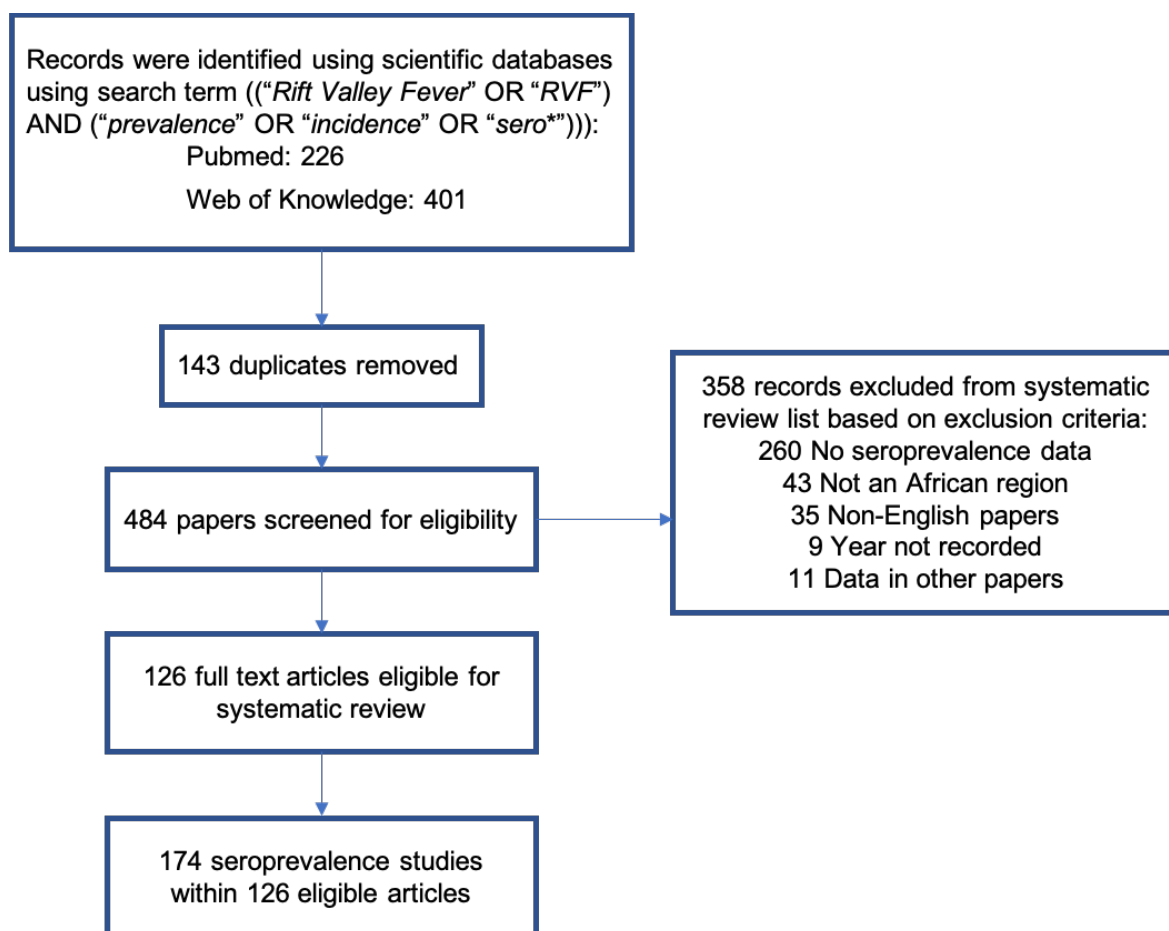


Figure 2.1 Flowchart for the systematic review to identify eligible studies of Rift Valley fever virus (RVFV) seroprevalence in Africa

Seroprevalence was analysed separately for goats, sheep, cattle, camels and humans. However, seroprevalence was pooled for wildlife due to the large range of species surveyed. The most common species tested was African buffalo (*Syncerus caffer*), but other species were sampled including elephants, white and black rhinoceros, giraffe, lions, leopards, lesser kudu, eland, kongoni, gazelle, impala, waterbuck and rodents (Anderson et al. 1998, Gora et al. 2000, Youssef et al. 2002, von Elm et al. 2007, Evans et al. 2008, LaBeaud et al. 2011, Miller et al. 2011, Caron et al. 2013, Fagbo et al., 2014, Lwande et al. 2015).

Where articles included multiple seroprevalence studies in different species or used different types of study design, each study was considered separately for the seroprevalence-specific analysis. However, articles reporting multiple seroprevalence studies, and specifically different study designs (e.g. cross-sectional and cohort), did not account for bias or diagnostic tests for each study individually and, therefore, the analysis of these aspects was conducted by article. Outbreak and inter-epidemic periods were classified according to the description in each individual article. If the period was not stated it was classed as inter-epidemic period.

Epidemiological study designs should account for bias where possible. Published in 2007, the STROBE statement is a valuable tool used to design and evaluate data analysis methods (Von Elm et al. 2007). Although eligible articles in this systematic review were published prior to the STROBE statement, these guidelines were used to provide a standardised assessment of the design and methods used for data analysis within the studies, as well as to assess the risk of different forms of bias in the study design (randomisation, recruitment, eligibility, exclusion), statistical power and statistical methods (Von Elm et al. 2007, Woolhouse et al. 2011).

RVFV seroprevalence distribution maps were created using the ggplot2 library (Wickham 2009) in R (version 3.3.3) (R Core Team 2013).

2.3. Results

A total of 126 articles met the inclusion criteria and reported RVFV seroprevalence (Figure 2.1). Seventeen of the articles included multiple seroprevalence studies using different species and types of study design. These were considered separately for the seroprevalence-specific analysis yielding a total of 174 studies. Many of the excluded studies were review articles, related to control policy or based on laboratory experiments.

2.3.1. Location and timing of RVFV seroprevalence studies in Africa

The earliest eligible study identified was carried out in 1968 (Davies 1975). Since then RVFV seroprevalence has been reported in 31 African countries (Figure 2.2). The focus of the majority of studies has been in eastern Africa with 48/174 (27.6%) seroprevalence studies being in Kenya alone (Figure 2.2).

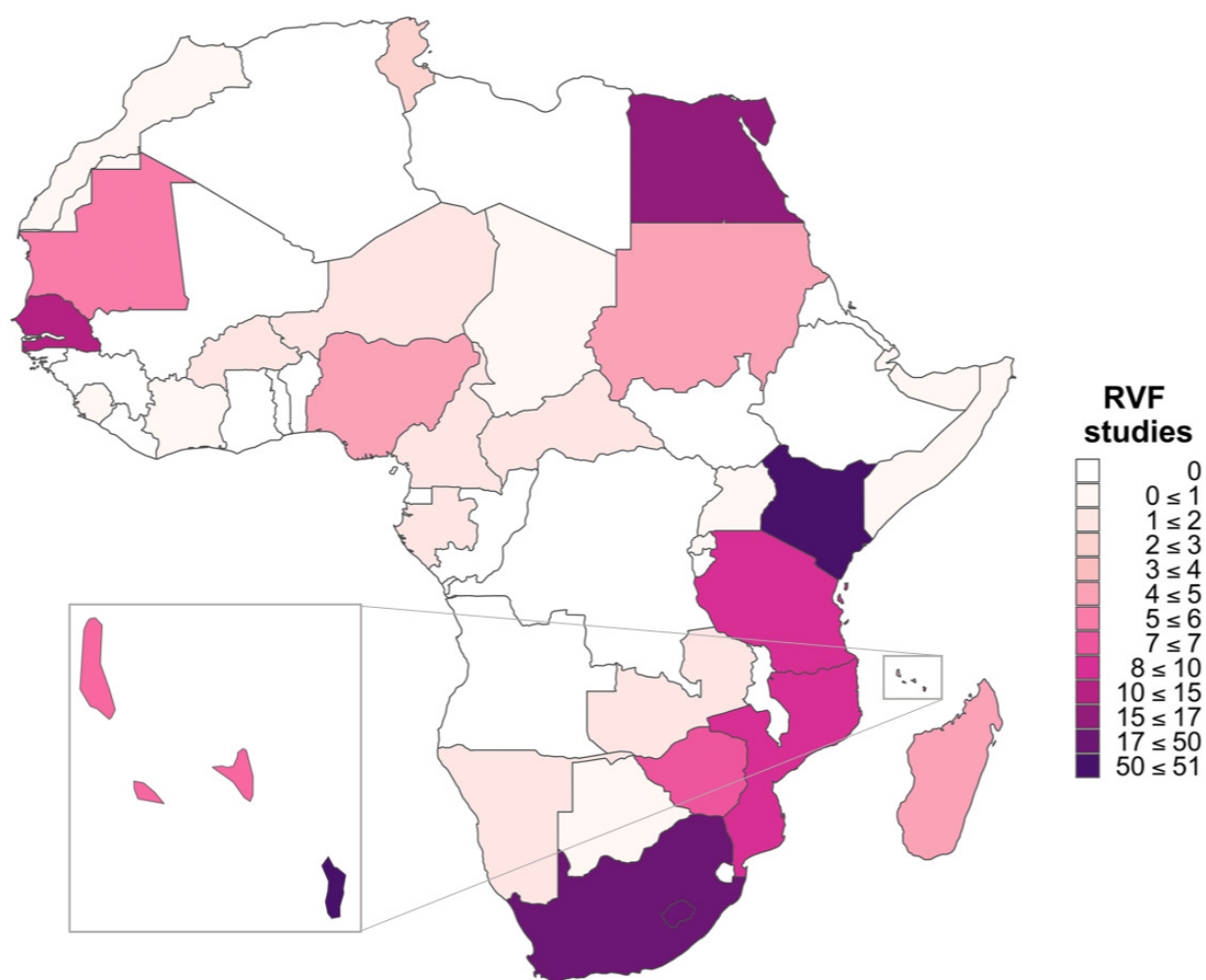


Figure 2.2 Number and geographical distribution of Rift Valley fever virus (RVFV) seroprevalence studies in African countries.

Although the first study included was conducted in 1968, there was a gap of over a decade before the next eligible study conducted in 1979 (Figure 2.3). Since 1979 seroprevalence studies have been carried out in most years (Figure 2.3). An increase in studies conducted for all species was evident in the 2000s (Figure 2.3). The peak years for studies were 2007 and 2010 during which reported outbreaks occurred (Himeidan et al. 2014). Of the 174 studies, 141 (81%) were conducted during IEPs, 32 (18%) during outbreaks and one (1%) immediately after an outbreak.

More RVFV seroprevalence studies have been conducted in livestock species (77/174 [44%]) than in humans (60/174 [34%]) or wildlife (40/174 [22%]). Over the last four decades 16/174 (9%) seroprevalence studies have been conducted in camels. All RVFV seroprevalence studies undertaken in wildlife species were conducted in IEPs and only in Zimbabwe, South Africa, Senegal and Botswana.

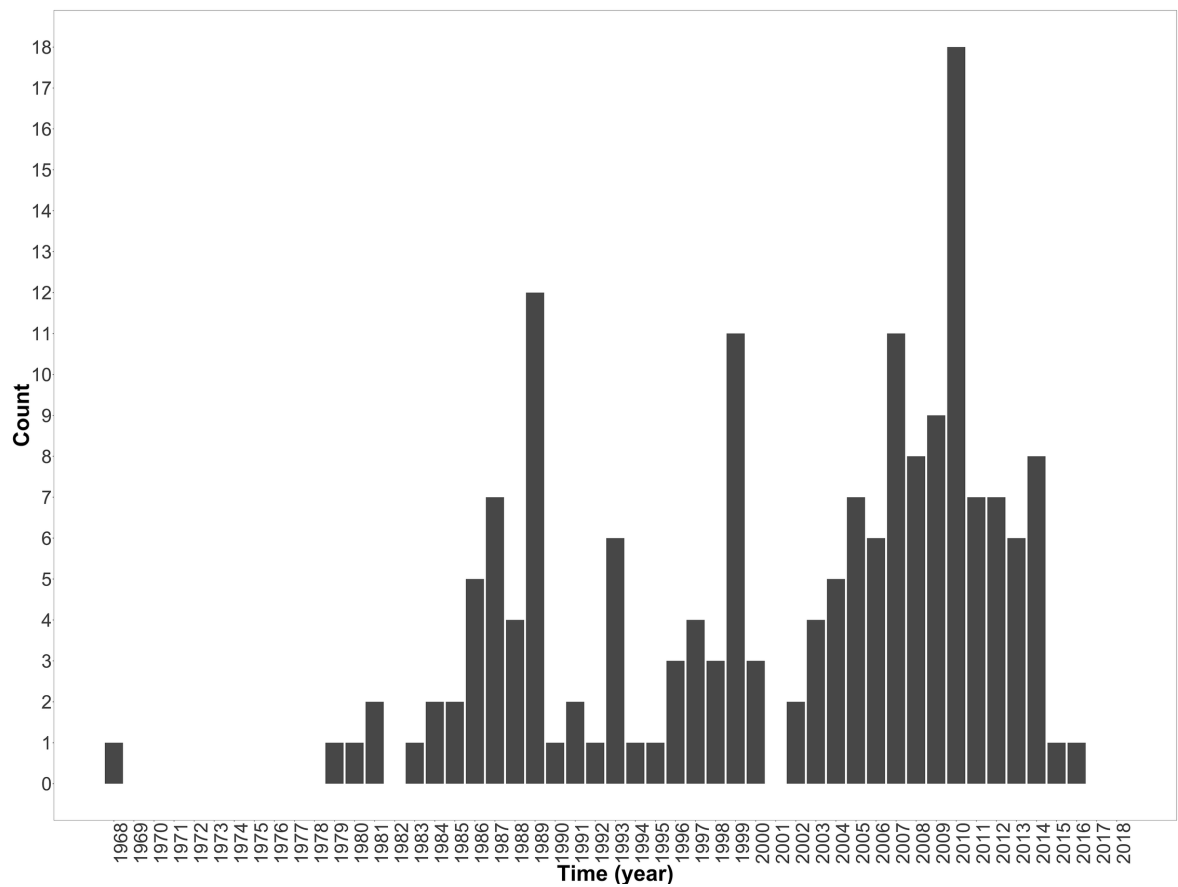


Figure 2.3 Number of Rift Valley fever virus (RVFV) seroprevalence studies conducted in African countries by year study was conducted.

2.3.2. Reported seroprevalence of RVFV

The seroprevalence of RVFV varied geographically and temporally in livestock, wildlife and humans. These trends are summarised in Figures 2.4 and 2.5, while the full data are presented in Appendix A2.2. Median RVFV seroprevalence was 12.9% in sheep (range 0-100%), 12.6% in cattle (range 0-100%); 11.3% in wildlife (range 0-87.5%), 10.1% in goats (range 0-69.6%); 8.8% in camels (range 0-57.1%) and 5.9% in humans (range 0-81.0%). The highest RVFV seroprevalence in livestock was identified in sheep and cattle in Egypt during an epizootic in 1997, where 100% of samples were seropositive (93 cattle and 57 sheep) (Appendix A2.2) (Abd el-Rahim et al. 1999). RVFV seroprevalence was significantly higher during outbreak periods compared to IEPs in goats (Wilcox rank sum test, outbreak median=50%, IEP median=9.4%, $p=0.001$) and sheep (Wilcox rank sum test, outbreak median=34.8%, IEP median=12.9%, $p=0.01$) but not in cattle, camels or humans (Figure 2.5).

Only six studies examined RVFV seroprevalence in livestock and humans concurrently, two studies examined seroprevalence in livestock and wildlife and no studies assessed wildlife and humans together (Table 2.1). Given the overall small number of concurrent studies, and limited information on links between species sampled, it is difficult to draw any conclusions about the relationship between RVFV seroprevalence in humans, wildlife and livestock.

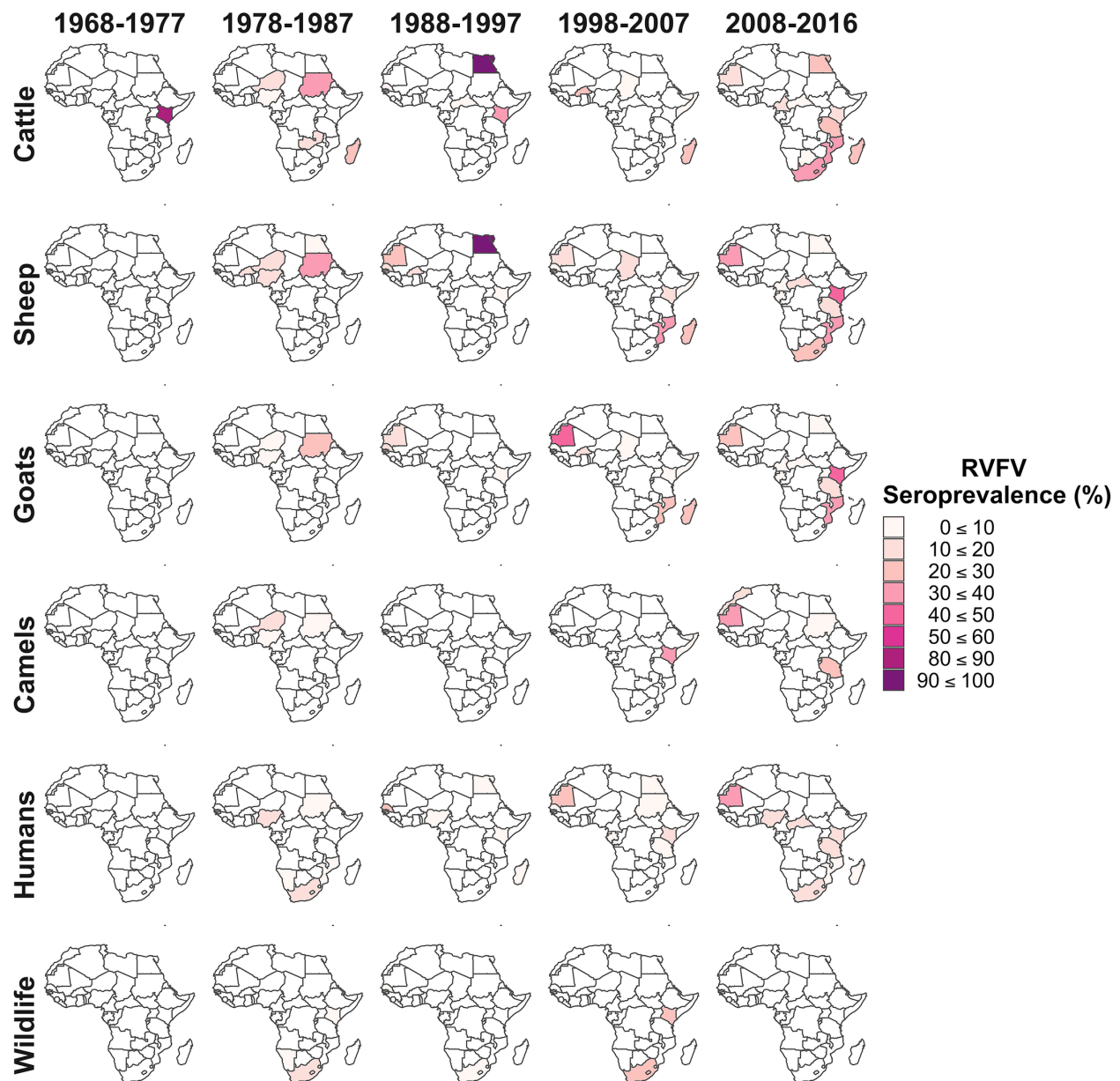


Figure 2.4 The distribution of seroprevalence (% individuals seropositive) for Rift Valley fever virus by species and decade in African countries, 1968-2016 (year study was conducted).

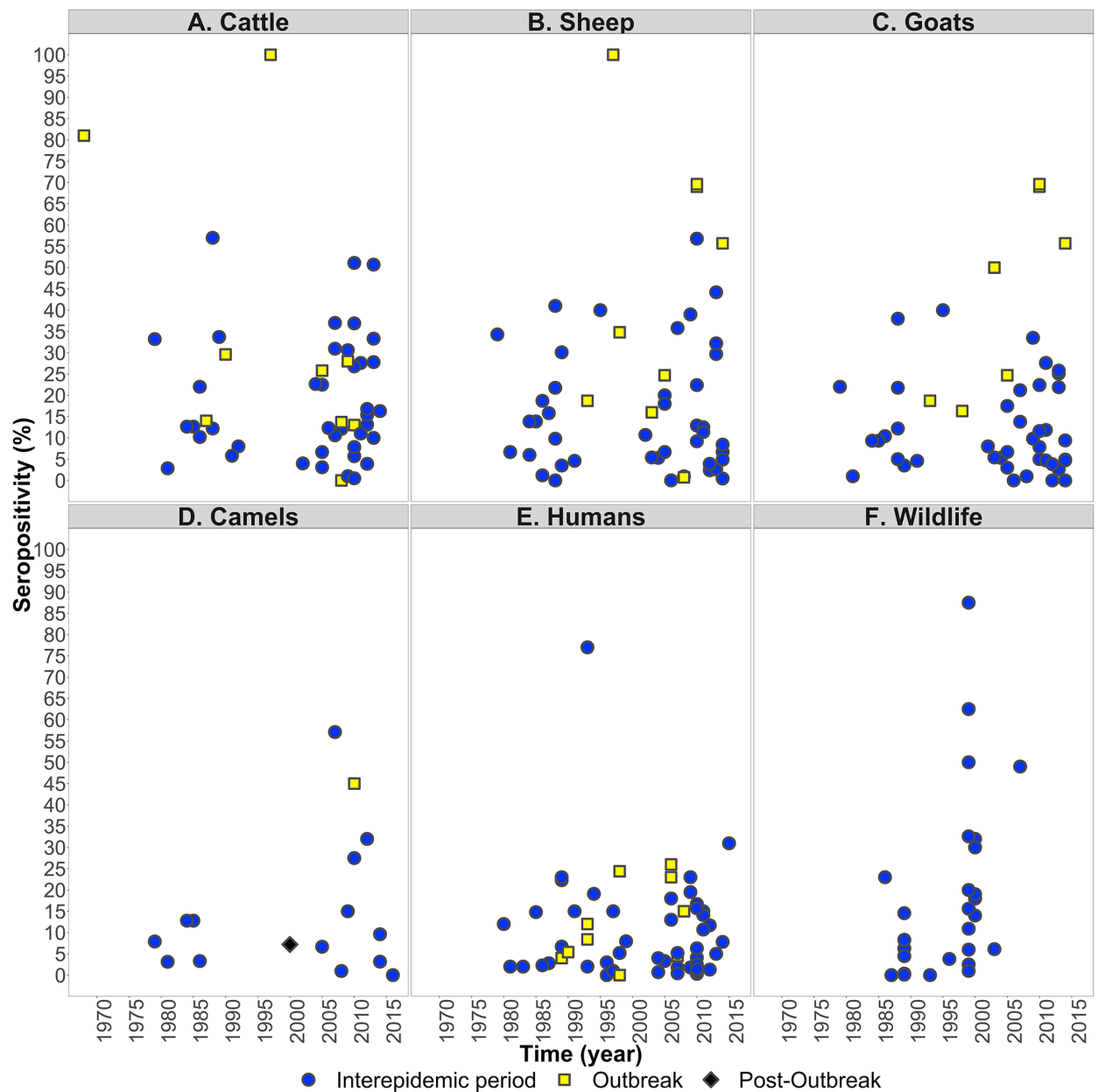


Figure 2.5 Reported seroprevalence (% individuals seropositive) of Rift Valley fever virus in (A) cattle, (B) sheep, (C) goats, (D) camels, (E) humans and (F) wildlife in African countries, during 1968-2016 (year study was conducted). Symbols indicate whether studies were reported as being carried out during outbreaks (filled yellow squares), immediately after outbreaks (filled back diamonds) or during inter-epidemic periods (filled blue circles).

Table 2.1 RVFV seroprevalence studies conducted in livestock, humans or wildlife concurrently.

Country	Year	African buffalo	% species seropositive				Link between populations	Ref.
			Humans	Goats	Sheep	Cattle		
Senegal	1989	-	22.3	-	30.1	-	Human samples were taken from sheep owners	Wilson 1994
Madagascar	1990	-	5.4	-	-	29.6	None reported	Morvan et al. 1991
Mauritania	1998	-	24.4	16.3	34.8	-	None reported	Nabeth et al. 2001
Central African Republic	2010	-	16.7	5.0	12.9	7.8	Samples taken from animals and humans at the same villages, livestock markets and slaughterhouses	Nakoune et al. 2016
Mayotte	2010	-	4.1	22.4	22.4	26.8	None reported	Lernout et al. 2013
Kenya	2010	-	1.4	-	-	0.5	Linkage was identified in some households sampled	Fevre et al. 2017
Zimbabwe	2008	5.3	-	-	-	12.1	Unfenced interfaces. Informal interviews stated buffalo are often seen at these sites	Caron et al. 2013
Botswana	2010	12.7	-	-	-	5.7	Cattle sampled based on proximity to protected area (where buffalo may be present)	Caron et al. 2013, Jori et al. 2015

2.3.3. Diagnostic tests

Information provided about the diagnostic test used varied considerably between articles (n=126): 25/126 (19.8%) did not state any details regarding diagnostic tests used; 64/126 (50.8%) described specific methods used; and 27/126 (21.4%) provided references to articles which described the test. Only 12 articles gave the diagnostic sensitivity and specificity of the tests (12/126 [9.5%]), seven of which used a commercial test (Nanyingi et al. 2016, Sumaye et al. 2015, Wensmann et al. 2015, Di Nardo et al. 2014, Sindato et al. 2015).

Commercial and in-house ELISAs were used in the majority of articles (94/126 [74.6%]). Virus neutralisation tests (VNTs) were used in a total of 32/126 articles (25.4%). In 17 of these 32 articles (53.1%) VNTs were used as a confirmatory test for the ELISAs, often only testing a subset of the samples.

2.3.4. Risk factors for RVFV seropositivity

In total, 41/126 eligible articles (32.5%) presented records of RVFV seroprevalence without any statistical analyses. The most common statistical method to compare seropositivity between groups used in the remaining articles was the univariate χ^2 test, which was the sole test used in 10/126 (8%) articles. Multivariate analysis was conducted in 23/126 (18.2%) of articles to establish whether multiple factors influenced RVFV seropositivity.

Several risk factors were significantly associated with seropositivity to RVFV in both humans and livestock, in particular increasing age (Tables 2.2 & 2.3). Occupations and practices associated with handling of animal blood/tissue were also identified as risk factors for seropositivity in humans (Table 2.2). Contradictory associations between risk and sex were reported, with some studies reporting an increased risk in males (Woods et al. 2002, Jeanmaire et al. 2009, Hassanain et al. 2010), some reporting a decreased risk in males (Owange et al. 2014, LaBeaud et al. 2007) and yet others finding no association (Blomstrom

et al. 2016). The seroprevalence of RVFV was significantly higher in sheep than in other species (Table 2.3) (Rostal et al. 2010, Lichoti et al. 2014, Nakoune et al. 2016 Blomstrom et al. 2016).

Table 2.2 Number of eligible articles in humans in Africa identifying potential risk factors as significantly associated with seropositivity for Rift Valley fever virus (RVFV) in final statistical model.

Risk factors for seropositivity to RVFV†	Number of articles using statistical methods (out of 51 eligible articles)		References
	Univariate	Multivariate	
Age ††	5	11	Morvan et al. 1991, Woods et al. 2002, LaBeaud et al. 2007, LaBeaud et al. 2008, Pourrut et al. 2010, LaBeaud 2011, Heinrich et al. 2012, Lernout et al. 2013, Andayi et al. 2014, LaBeaud et al. 2015, Tigoi et al. 2015, Sumaye et al. 2015, Dellagi et al. 2016, Cook et al. 2017, Grossi-Soyster et al. 2017
Sex*	2	5	Woods et al. 2002, LaBeaud et al. 2007, LaBeaud et al. 2008, Hassanain et al. 2010, Pourrut et al. 2010, Lernout et al. 2013, LaBeaud et al. 2015
Occupation**	1	2	Olaleye et al. 1996, Anyangu et al. 2010, Cook et al. 2017
Contact with livestock foetus	0	2	LaBeaud et al. 2008, Anyangu et al. 2010

† risk factors were only included in this table if they were found to be significant in at least two articles

††RVFV seroprevalence increased with age

*sex was identified as a risk factor for RVFV seropositivity in a number of studies, but the conclusions were contradictory (see text)

**occupations involving contact with animal blood and products were associated with an increased risk

Table 2.3 Number of studies on livestock in Africa identifying potential risk factors as significantly associated seropositivity for Rift Valley fever virus (RVFV).

Number of articles using statistical methods (out of 70 eligible articles)			
Risk factors for seropositivity to RVFV [†]	Univariate	Multivariate	References
Age ^{††}	7	8	Wilson et al. 1994, Rostal et al. 2010, Abdallah et al. 2011, Chevalier et al. 2011, El-Harrak et al. 2011, Jeanmaire et al. 2011, Roger et al. 2011, Fafetine et al. 2013, Boussini et al. 2014, Sindato et al. 2015, Swai et al. 2015, Blomstrom et al. 2016, Nakoune et al. 2016, Nanyingi et al. 2017, Umuhoza et al. 2017
Species	5	1	Rostal et al. 2010, Boussini et al. 2014, Kifaro et al. 2014, Lichoti et al. 2014, Blomstrom et al. 2016, Nakoune et al. 2016
Sex*	1	1	Jeanmaire et al. 2011, Owange et al. 2014
Animal introduced into herd	1	1	Sindato et al. 2015, Swai et al. 2015
Nearby water point	0	2	Chevalier et al. 2011, Lernout et al. 2013

[†] risk factors were only included in this table if they were found to be significant in at least two articles

*sex was identified as a risk factor for RVFV seropositivity in a number of studies, but the conclusions were contradictory (see text)

^{††}RVFV seroprevalence increased with age

2.3.5. Assessment of biases in RVFV seroprevalence studies

The majority of studies in the systematic review were cross-sectional (a study design which assesses a population at one point in time) (149/174 [85.6%]); 20/174 (11.5%) were longitudinal studies (a study design which assesses the same population at multiple time points); 3/174 (1.7%) were case-control studies (a study design which compares two groups differing in outcome and compared on the basis of supposed exposures) (Rift Valley fever-Egypt, 1994, Jackel et al. 2013); and 2/174 (1.1%) were cohort studies (a study design which is a type of longitudinal study conducted on a population, following a group of individuals/animals that share a common characteristic, studies are performed as cross-sections at different time intervals) (Heinrich et al. 2012, LaBeaud et al. 2011). Few of the eligible articles (n=126) in this systematic review accounted for bias (Table 4) and only one article (Mease et al. 2011) accounted for all five potential sources of bias outlined by the STROBE statement (Table 4) (Von Elm et al. 2007). Eligibility and exclusion criteria as well as power calculations were seldom reported in the articles. A range of random sampling schemes were used (Thiongane et al. 1991, Gonzalez et al. 1992, Ringot et al. 2004, Mariner et al. 1995, Ayari-Fakhfakh et al. 2011, Roger et al. 2011, Caron et al. 2013, Lernout et al. 2013, Sumaye et al. 2013, Boussini et al. 2014, Odendaal et al. 2014, Owange et al. 2014), although the majority of articles did not specify the randomisation protocol (94/126 [74.6%]). Vaccination status of livestock was not reported in 37/51 (72.5%) livestock seroprevalence eligible articles.

Table 2.4 Number of Rift Valley fever virus seroprevalence articles in Africa accounting for risk of bias outlined in the STROBE statement

Species	Randomisation	Recruitment	Exclusion	Eligibility	Power calculation for sample size
Livestock	20	35	2	5	13
Humans	13	31	8	18	6
Livestock and Humans	1	1	1	2	0
Wildlife	0	8	0	0	0
Total (% of 126 eligible studies)	34 (27)	75 (59.5)	11 (8.7)	25 (19.8)	19 (15.1)

2.4. Conclusions

Over the last four decades there has been an increasing number of RVFV seroprevalence studies carried out in wildlife, livestock and humans in Africa (Figure 2.3). There have been a number of RVFV outbreaks during the 2000s, which may have resulted in an increase in RVF awareness and resources for surveillance leading to an increase in seroprevalence studies during IEPs (Figure 2.3). There is, however, limited geographical coverage of RVFV studies: some countries in North and East Africa have not reported outbreaks or assessed seroprevalence (Figure 2.2 & 2.4). This is similarly seen on WHO and CDC distribution maps which account for case finding and isolation of virus (CDC, 2020). This is relevant from an epidemiological perspective since RVFV has been detected in other countries within these regions, thus increasing the risk of viral incursions through animal trade, which has been implicated as the main route of virus dissemination in several outbreaks (Cetre-Sossah et al. 2012, Lernout et al. 2013, Roger et al. 2014, Napp et al. 2018). Evaluation approaches identify that camel trade into (from Sudan) and within Egypt is a main risk of RVFV transmission into the country with animal markets and slaughterhouses being sites where risk of RVFV transmission was high (Napp et al. 2018). The risk posed by animal trade also suggests that cross-border surveillance in both livestock and humans is required to add to the understanding and significance of this route for RVFV transmission.

A number of countries have conducted very few seroprevalence studies (Figure 2.4; Appendix A2.2) which makes it difficult to assess temporal trends. In those countries where more studies were conducted, temporal trends are indicative of recurrent outbreaks (for example: Comoros, Mayotte, Kenya, Senegal or Mauritania) (Appendix A2.2). However, caution is warranted when identifying trends from a series of cross-sectional studies. When studies are undertaken in different districts of a country, and thus different environments, this may have an impact on viral maintenance, transmission, and, hence, seropositivity and

so limits generalisability at a country level. Similarly, in Kenya several studies were conducted on the same species in the same year, but variation in seroprevalence within years may be indicative of specifics to the study (location, age of animals/humans, study design, tests used).

The higher RVFV seroprevalences seen in animals compared to humans (Figure 2.5) may be a consequence of differing forces of infection (i.e. transmission only occurring as a spillover event in humans and thus risk of transmission is much lower). The significantly higher seroprevalence during outbreak years compared to interepidemic years seen in sheep and goats, but not in other species, is likely due to the faster population turnover in sheep and goats, which means there is a bigger pool of susceptibles to become infected between IEPs and outbreak periods. It has been suggested previously that wildlife species may act as reservoirs of the virus. The high RVFV seroprevalence recorded in wildlife during IEPs supports this hypothesis and provides a basis that low-level circulation may be taking place in these species (Olive et al. 2012).

The lack of seroprevalence studies in wildlife over the last decade suggests this is a neglected area of research. Such studies are essential for further understanding the role of wildlife in viral maintenance and potential spillover into livestock animals, particularly during outbreak periods. Indeed, to understand the impact of cross-species transmission, seroprevalence studies should ideally be conducted concurrently in livestock, wildlife and humans. This would allow the force of infection between species to be estimated and, hence, the relative importance of different species in the transmission dynamics of RVFV. Yet the results of this systematic review indicate that such studies are seldom carried out and, even when they are, the relevant linkages between the species are not recorded (Table 2.1). A complete picture of cross-species transmission would also require consideration to be given to mosquito species present and their host feeding preferences (Linthicum et al. 2016).

A number of risk factors for human RVFV seropositivity were associated with contact practices with livestock (Table 2) and further research on risk factors in livestock may indicate measures that could reduce spillover of infection into humans. A pragmatic approach would seek to understand virus maintenance, especially within the environment, vectors and wildlife populations to aid in targeting potential risk factors of RVFV seropositivity in livestock. RVFV-induced abortions are a recognised clinical sign of the disease in livestock and, recently, a study has found associations between miscarriages in women and infection with RVFV (Baudin et al. 2016). This demonstrates the need for further research and identifies another at-risk population.

The true seroprevalence of RVFV is often uncertain due to three factors affecting interpretation of published seroprevalence studies. First, most studies do not provide enough information on the diagnostic tests used, including their sensitivity and specificity. If this information is provided it is possible to estimate the true prevalence (i.e. allowing for false positive and false negative results) from the apparent prevalence (Thrusfield et al. 1995). As a commercial assay, the ID Screen® RVFV competition multispecies ELISA (ID-Vet, Montpellier, France) used in many of the studies was validated by carrying out a ring trial, which demonstrated that the test has a high specificity (100%) and sensitivity (ranged from 91-100%) (Kortekaas et al. 2013). An inhibition ELISA was also commonly used which has been shown to provide 100% sensitivity, and 99.29% and 100% specificity in sheep and camels, respectively (Paweska et al. 2005). This suggests that for these tests there is a low probability of false positives or false negatives and, hence, the apparent prevalence will be a reasonable approximation of the true seroprevalence. However, reporting the diagnostic test used and its sensitivity and specificity would be a recommendation for future RVFV prevalence studies. Second, there are currently no commercial vaccines that are compliant with tests that are able to differentiate between vaccinated and infected animals (DIVA),

meaning vaccinated animals will be classified as seropositive and, hence, the true seroprevalence could be overestimated. Thus, vaccination history of recruited animals needs to be recorded in the study design. Third, antibody responses to RVFV infection are long lived with RVFV-specific antibodies having been reported in humans over 12 years after the only known exposure (Sabin et al. 1947, Brown et al. 1957). Consequently, serological assays are unable to confirm when exposure took place (Smithburn 1949a, Zeller et al. 1995, Smithburn et al. 1949b). Two possible ways of assessing the level of recent infection would be through IgM ELISAs (IgM antibodies are short-lived for RVFV, which would be ideal for showing endemic virus circulation) (Niklasson et al. 1984) or through use of RT-PCR to detect RVFV RNA (Wilson et al. 2013).

This review has highlighted the potential for bias in the designs of many RVFV seroprevalence studies, further complicating their interpretation. In many studies there was a lack of randomisation, recruitment criteria or insufficient sample sizes. Recruitment is important in understanding disease transmission in livestock, particularly with regard to animal trade histories and where animals have been exposed to the virus prior to the study (e.g. somewhere other than the study locations). Many studies omitted information on livestock characteristics such as age, sex and breed, all of which are factors that could influence interpretation of RVFV seroprevalence results. There is a need to improve study design reporting to provide validity, transparency and reproducibility. By implementing standardised methods, data can be objectively examined and compared (Kilkenny et al. 2009, Sena et al. 2014).

The RVFV seroprevalence distributions visualised in the maps were pooled by country and decade (Figure 2.1 and Figure 2.4). These maps provide an overview of seroprevalence on the African continent, showing the variation both between countries over the same time period and within countries over different time periods. However, it should be borne in mind

that this could mask heterogeneity in seroprevalence within a country, which reflects processes at smaller geographical, spatio-temporal and epidemiological scales (Clements et al. 2007) (for example within year variation of seroprevalence in Kenya due to sampling in different regions of the country). Pooling the wildlife species introduced a bias in itself, but this was done to provide a representation of what seroprevalence is seen in wildlife, particularly as little research has been done on this. This systematic review followed PRISMA guidelines (Moher et al. 2009) to provide a comprehensive unbiased overview and collection, although some articles may have been missed due to only including studies published in the English language (Figure 2.1).

One third of articles included in this systematic review did not conduct any statistical analysis of the data they generated. This limits their usefulness in terms of both supporting evidence-based decision making and furthering the understanding of the disease. Other studies have identified risk factors associated with seropositivity to RVFV (Table 2 and 3). However, the level of significance (e.g. cut off p-values) required for something to be deemed a risk factor varied across articles. Moreover, there were conflicting reports of statistical significance for risk factors amongst studies. This could be explained by: the heterogeneity of study design; the complex nature of different environments and the impact this has on the ability of the virus to transmit; the methodology of the operator conducting the diagnostic tests; or those that may have occurred by chance.

The contradictory relationship with sex and the risk of seropositivity may reflect differences in local culture surrounding gender and animal handling/management practices. Understanding the social aspect of disease transmission could provide valuable information to disease spillover dynamics. The risk of seropositivity increased with age in both humans and livestock. This most likely reflects the increase probability of exposure with age rather than implying age-dependent susceptibility. Where the risk of seropositivity was found to

differ significantly amongst livestock species, sheep were at increased risk in a number of studies, again presumably due to the high population turnover, and also differences in susceptibility or host immune response, vector host preference or animal management practices (Rostal et al. 2010, Blomstrom et al. 2016), though some studies found no difference between species (Rostal et al. 2010, Lichoti et al. 2014). Finally, the risk of seropositivity in livestock increased with proximity to water points (Lernout et al. 2013) and, hence, presumably to vectors. Another study found contradictory results, but this was explained by cattle movement back to pens during early evening which is where exposure to mosquitoes and, hence, RVFV was believed to take place (Chevalier et al. 2011).

Since completing this systematic review RVFV seroprevalence studies have continued to be conducted and published. These papers have not been included in the analysis due to the explicit time frame for conducting the searches recorded in the methods. However, it is important to report that seroprevalence studies have been conducted in countries that had not reported RVFV seroprevalence before and seropositivity confirmed, during interepidemic periods. Mali has reported RVFV seropositivity in cattle (Subudhi et al. 2018) as well as positive cases in humans (Tong et al. 2019). The Democratic Republic of Congo (DRC) has also confirmed RVF seropositivity in cattle (Halawi et al. 2019).

This chapter sought to highlight important trends and gaps in RVFV seroprevalence in Africa and address where the risk factors lie in cross-species transmission events. In particular, few studies to date have sampled humans, wildlife or livestock species concurrently. This demonstrates that future medical and veterinary research should adopt a “One Health” approach by performing concurrent livestock and human studies, as well as assessing spillover events between wildlife and livestock. Understanding the epidemiology and immunology of RVFV in different species will aid targeting control measures such as

vaccination to priority species (Lorenzo et al. 2015) and geographical areas, helping livestock workers in their economic and health prosperity.

3. Seroprevalence of Rift Valley fever virus in Kenya

3.1. Background

The mechanisms by which RVF is maintained in the environment during long periods between outbreaks are yet to be fully elucidated. One way to address this question is to analyse RVFV antibodies in livestock species with known ages during these IEPs. By assessing RVFV antibodies in livestock, results can support understanding of virus maintenance in the environment; transmission in the absence of reported cases; and the intensity of this specific to livestock species of interest. It can also help identify potential settings for spillover into human populations. This chapter analyses primary and secondary RVFV seroprevalence data in livestock in multiple Kenyan counties across different years. Outbreaks in livestock often precede occurrence of RVF in humans. It is well known that the main route of transmission from animals into humans is through direct contact with infected tissues and blood. Humans are at 2.23 increased odds of exposure if at least one of their animals is seropositive to RVFV (Bett et al. 2019). There has been a substantial amount of research published on RVF seroprevalence in wildlife, livestock and humans in Kenya over multiple years, as discussed in Appendix A2.2. The systematic review (Chapter 2) found a range of RVFV seroprevalence values in different livestock and wildlife species ranging from 0 – 87.5% from 1968 – 2014 in the country (Clark et al. 2018). This chapter reports and analyses RVFV seroprevalence in livestock species from 2013 – 2019.

Although RVFV was discovered in Kenya, the vast and variable environment of the country means conducive conditions for virus maintenance are complex. Risk maps for RVFV in Kenya have been developed to identify regions of low, medium and high risk of RVF transmission. These assess risk by modelling key environmental indicators that precede outbreaks and presumably make it conducive for *Aedes* mosquitoes (RVFV primary vector) survival (Linthicum et al 1999, Anyamba et al 2009, Britch et al 2013). Risk maps also utilise data from previous outbreaks to identify where at-risk areas are based on proportion of the

total outbreak years (exposure) and severity of disease in humans and animals (outcome) (Munyua et al 2016). There are limitations to these models; mainly the amount of serological data available over multiple time frames and in different counties of the country. These maps can therefore draw conclusions based on history but their usefulness in assessing emergence is questionable. Traditional climatic patterns in East Africa and across the rest of the world are shifting and in recent years small localised outbreaks of RVFV have been emerging (OIE, 2019) (albeit potentially due to increased surveillance). This therefore warrants continued sero-surveillance across different counties and environments, specifically those that have previously been reported as low or medium risk areas as climate and environments change, specifying precise locations for where surveillance has been conducted (Britch et al. 2013).

In this chapter RVF seroprevalence is assessed in four livestock species in three different counties of Kenya: Kisumu, Machakos and Laikipia (Fig 3.1). These three counties are distinct in environmental indicators and previous risk levels for RVFV.

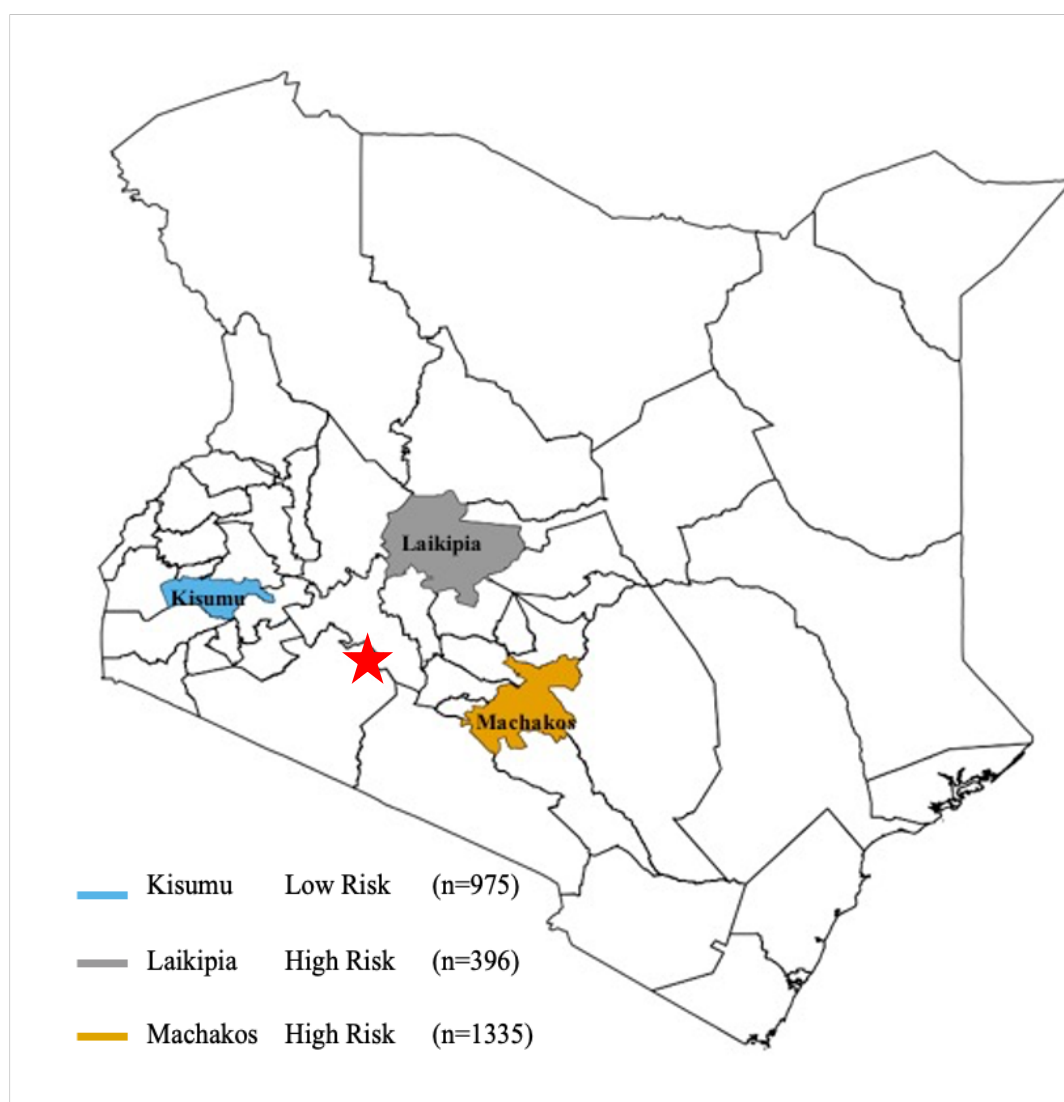


Figure 3.1 Map of Kenya with three counties where RVFV seroprevalence was measured

Kisumu (Blue), Laikipia (Grey), Machakos (Yellow), risk areas and number of animals sampled. Naivasha, where RVFV was first discovered is shown by a red star.

Kisumu county is classified as a tropical climate with a significant amount of rainfall (~1321 mm annually); its altitude is 1131m above sea level. The main city, Kisumu is located on the shores of Lake Victoria (Figure 3.2). RVFV transmission in Western Kenya is believed to be low and RVFV outbreaks have not been reported (Murithi et al 2011, Cook et al 2017). Human seroprevalence studies conducted in western Kenya in 2012 have seropositive estimates ranging from 0 – 3% (Cook et al 2017). Evidence of RVF transmission in livestock in this area is lacking, but it is hypothesised that this is likely to be low too. Although a low risk area, the primary vectors, *Aedes* spp. have been found in the county, as have secondary vectors, including *Mansonia* spp. and *Culex* spp. (Lutomiah et al 2013).

Machakos is a county southeast of Nairobi that has been classed as a high-risk area for RVFV based on putative risk factors and previous outbreaks. Since 1961 it has had over 21 outbreaks of RVFV (Murithi et al 2011) and the virus is believed to be enzootic in this area. The county is predominately semi-arid land and is approximately 1650-1900m above sea level (Figure 3.3). It has approximately 500mm of rain yearly. During El Niño rains in 2015-2016 in Kenya enhanced surveillance was conducted in multiple counties including Machakos. Despite being classified as a high-risk area no seropositive samples were recorded in 102 sheep tested in the county (Oyas et al 2018).

Laikipia county is semi-arid and is made up by a number of conservancies to protect the local wildlife and environment, allowing for co-existence between wildlife and livestock (Figure 3.4). Altitude ranges from 1500m to 2611m above sea level and has approximately 972mm of rainfall annually. RVFV seroprevalence and surveillance studies in multiple species have been conducted in Laikipia (Evans et al 2008, Britch et al 2013, Lwande et al 2015). A total of 23 outbreaks have been reported since 1951 in the county, a high-risk area for RVFV (Murithi et al 2011, Munyua 2016). A study assessing prevalence of antibodies to RVFV in wildlife found that 37.5% (3 out of 8) of buffalo tested in the county were

seropositive to RVFV antibodies. Further studies conducted on wildlife in the region found that 12.5% (2 out of 16) wildlife species tested positive to RVFV antibodies between 2008-2015 with no significant difference between years (Lwande et al 2015). In 2014 cattle were sampled on a conservancy within Laikipia. Cattle were in contact with wildlife species including buffalo, though all cattle were IgG seronegative to RVFV (Lwande et al 2015).

This study assessed RVF seropositivity by testing IgG seroprevalence to RVFV in four species, sheep, goats, cattle and camels. Antibodies to RVFV have previously been isolated in multiple Kenyan counties in all four species (Britch et al 2013).



Figure 3.2 Lake Victoria in Kisumu 2017



Figure 3.3 ILRI Kapiti Ranch, Machakos 2019

Photo credit Danny Wright



Figure 3.4 Laikipia March 2018

3.2. Methods

3.2.1. Sample acquisition

Opportunistic testing was carried out on samples from the Kisumu county (Figure 3.6) taken from livestock that were a part of an ongoing population-based animal syndromic surveillance system (PBASS) which is linked with a human health, population-based infectious disease surveillance platform (PBIDS) based in western Kenya (Thumbi et al. 2015). This system monitors health of livestock animals by collecting data on syndromes including: reproductive, respiratory, digestive, urogenital, musculo-skeletal, skin, nervous and udder disorder, as well as death. A subset of samples were randomly picked from each year from 2013 to 2016 and from different species (sheep, goat and cattle) (Table 3.1). As these samples were tested ad hoc from a surveillance system, there were no eligibility or exclusion criteria, nor were power calculations undertaken. The history of RVFV was unknown in these animals.

Table 3.1 Number of animals sampled per year from the PBASS surveillance system in Kisumu

	2013	2014	2015	2016	Total
Sheep	66	88	41	42	237
Goats	95	93	87	35	310
Cattle	106	193	97	32	428
Total	267	374	225	109	975

Samples tested for RVF seropositivity from Machakos and Laikipia were collected and analysed as part of a recruitment study for the vaccine field trial reported in chapter 5. Recruitment followed criteria outlined by the vaccine field trial protocol (see chapter 5). Animals sampled were between the ages of four months and four years to avoid maternal antibodies. A total number of 200 animals per species (cattle, sheep and goats), or all available samples from Dromedary camels. The field trial needed 180 animals per species, we tested 200 in case any animals needed to be replaced because they were RVF seropositive. Due to delays in vaccine development, recruitment was conducted twice over two consecutive years (2018 and 2019) to confirm animals entering the vaccine trial were still seronegative to RVFV.

The ILRI Kapiti Plains Ranch Ltd. is situated in the Machakos county (1.63°S, 37.15°E) (Figure 3.6). It has approximately 2900 cattle (Boran and Boran/Friesian crosses), 1400 sheep (Red Masaai, Red Masaai/Dopler crosses) and 500 goats (Galla) on approximately 33,000 acres of semi-arid land. The farm practises outdoor grazing with hay supplementation when needed. Wild animals are able to move through the property as there is no fence on the southern border of the ranch. Such wildlife include wildebeest (*Connochaetes spp.*) (Figure 3.5), giraffe (*Giraffa camelopardalis tippelskirchii*), hartebeest (*Alcelaphus buselaphus*), zebra (*Equus quagga*), Thomson's Gazelle, (*Eudorcas thomsoni*) Kudu and impala (*Aepyceros melampus*), as well as lions (*Panthera leo*), hyena (*Crocuta crocuta*), leopard (*Panthera pardus*) and cheetah (*Acinonyx jubatus*). Although situated in the Machakos county, the ILRI Kapiti farm has never reported an RVF outbreak. For this study sheep, goats and cattle were sampled on this farm.



Figure 3.5 Livestock on the Kapiti Plains Ranch Ltd are in frequent interaction with wildlife animals, such as wildebeest.

In the Laikipia county goats were sampled on one farm and camels on three farms (Figure 3.6). Galla goats were sampled on the Ol Doinyo Lemboro farm which covers 20,000 acres (0.47°S 37°E). The farm also has cattle and sheep. Goats are vaccinated against contagious caprine pleuropneumonia and grazed outdoors. Camels were sampled on the Suyian Ranch (0.49°N, 36.82°E), Loisaba Ranch (0.61°N, 36.83°E) and Mpala Research Centre (0.29°N, 36.90°E). Sampling was undertaken in March 2018 and March-April 2019.

During the 2019 sampling a pilot study was conducted in a small subset of animals to test efficacy of the Smithburn vaccine (not part of this thesis). The sheep, goats and cattle were already owned by the Kapiti farm (where the vaccine trial was conducted), but the camels came from ranches in Laikipia. As a subset of the camel herd would be vaccinated with the Smithburn vaccine (n=5), transmission from vaccinated camels that may be shedding the vaccine virus was checked. As these vaccinated camels were followed up for a month it was decided all camels in the herd would be re-tested as a longitudinal study to check for any sero-conversion that may be due to virus shedding following vaccination with the Smithburn vaccine.

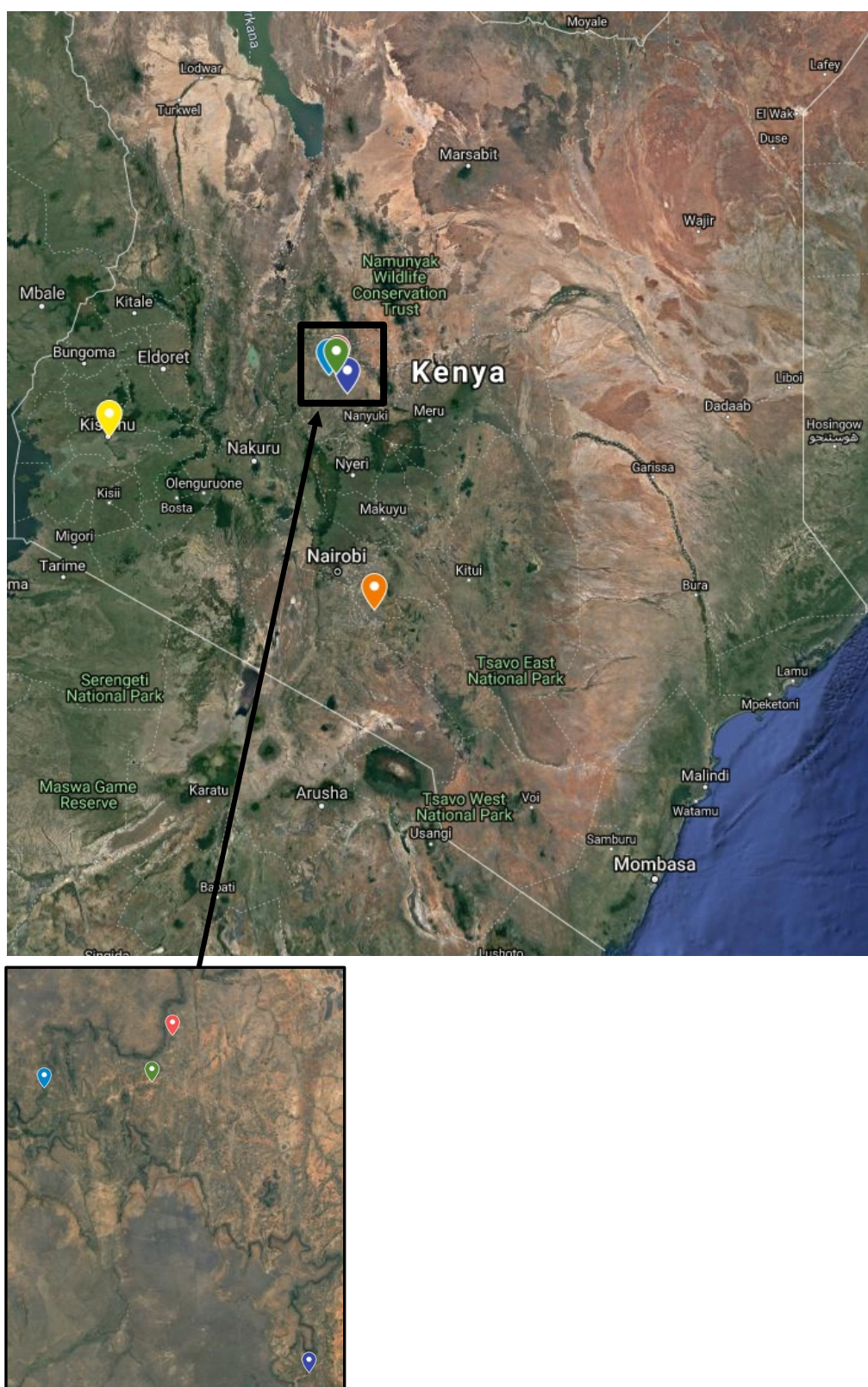


Figure 3.6 Map of areas sampled in Kenya

Kisumu (Yellow), Kapiti, Machakos (Orange) Loisaba Ranch, Laikipia (Red), Mpala Ranch, Laikipia (Purple), Suyian Ranch, Laikipia (Blue), Ol Doinyo Lemboro Ranch, Laikipia (Green). Google maps accessed 20/11/2019.

3.2.2. Sample Collection

Ethical approval to collect samples was gained under the wider vaccine study from The ILRI Institution Animal Care and Use Committee (IACUC-RC2018-07), KEMRI-Wellcome Trust Programme and The Pirbright Institute (see Chapter 5).

Sample acquisition details for the Kisumu PBASS samples can be found in published literature, although specifics on where blood was taken from the animal are not recorded (Thumbi et al. 2015). For samples taken from Machakos and Laikipia, blood was collected from the jugular vein in all animals in a 10ml plain vacutainer tube (Becton Dickinson) (Figure 3.7). Blood was spun down in a centrifuge at 3000rpm for 20 minutes and two aliquots of 1.2ml serum were taken for each animal. Samples were stored in liquid nitrogen during the Laikipia trip and put into long term storage at -80°C once transported to KEMRI-Wellcome Trust Programme in Kilifi.



Figure 3.7 Camel sampling at the Loisaba ranch, Laikipia 2018

3.2.3. ID Screen® ELISA

The ID Screen® RVFV competition multispecies ELISA (ID Vet; Montpellier, France) was used to screen animals for RVFV IgG antibodies directed against the nucleoprotein of the RVF virus. Briefly, wells were pre-coated with a recombinant RVFV nucleoprotein, samples and controls were added to the wells and, if antibodies were present in the serum samples, they formed an antibody-antigen complex which masked the nucleoprotein epitopes. Anti-nucleoprotein-peroxidase (HRP) conjugate was added to the wells and bound to any remaining free nucleoprotein epitopes as an antigen-conjugate. Washing removed any excess conjugate and TMB substrate was added. If antibodies were present there was no colour, if antibodies were not present the wells appeared blue which became yellow after adding stop solution. The ELISA kit has a sensitivity of 98.4% and specificity 100% (Kortekaas et al. 2013). Optical readings were performed at 450nm. The competition percentage (S/N%) was calculated as: OD samples were divided by the average of the negative control x 100. Samples with S/N% of less than or equal to 40% were considered positive, greater than 40% and less than or equal to 50% were considered doubtful and greater than 50% were considered negative.

3.2.4. Statistical analysis

Statistical analysis was conducted on the MASS package (Venables and Ripley 2002). The ggplot2 package (Wickham 2016) of the R statistical software environment (version 3.3.3) (R Core Team 2013) was used to produce Figure 3.1 and google maps was used to produce Figure 3.6. Fisher's exact test was used to assess differences in RVFV seropositivity between male and female animals.

3.3. Results

3.3.1. Cross-sectional sampling

Results from cross-sectional samples taken between 2013-2016 show there is minimal circulation of anti-RVSV antibodies in sheep, goats and cattle in the Kisumu county (Table 3.2). None of the goats sampled were seropositive to RVSV during this time, two cattle were RVSV seropositive in 2015 and one sheep was seropositive in 2016.

No circulation of RVSV antibodies was reported in sheep in 2018 or 2019 in Machakos, two goats were seropositive, one in 2018 and another in 2019. One herd of cattle at the Kapiti ranch had 5.4% seropositivity in 2018, while a separate herd, sampled in 2019, had a lower seroprevalence of 2%. The herd of cattle sampled in 2018 were aged between 7 and 14 months old and so were born after the last known outbreak in the country (2007). There was a significant difference in seropositivity between male or female cattle ($p=0.02$) with males having a higher seroprevalence. The highest record of seroprevalence was in a herd of Galla goats in Laikipia in 2018, with 17.9% being seropositive. The seroprevalence in goats was significantly ($p=0.005$) higher in female goats than males (Table 3.3).

Table 3.2 RVFV Seroprevalence in livestock species 2013 – 2019 a cross -sectional study

Location	Species	Number Seropositive	Number sampled	Year
Kisumu	Cattle	0 (0)	106	2013
	Goats	0 (0)	95	2013
	Sheep	0 (0)	66	2013
	Cattle	0 (0)	193	2014
	Goats	0 (0)	93	2014
	Sheep	0 (0)	88	2014
	Cattle	2 (2.1)	97	2015
	Goats	0 (0)	87	2015
	Sheep	0 (0)	41	2015
	Cattle	0 (0)	32	2016
	Goats	0 (0)	35	2016
	Sheep	1 (2.4)	42	2016
	Cattle	11 (5.4)	205	2018
	Sheep	0 (0)	200	2018
	Goats	1 (0.8)	132	2018
Machakos (ILRI Kapiti)	Sheep	0 (0)	199	2019
	Cattle	2 (1.0)	207	2019
	Goats	1 (0.5)	200	2019
	Goats	35 (17.9)	195	2018
	Goats	35 (17.9)	195	2018
Laikipia (Ol Doinyo lemboro farm)	Camels	0 (0)	89	2018
	Camels	0 (0)	74	2019
	Camels	13 (34.2)	38	2019 (March)
	Camels	3 (11.1)	27	2019 (April)

Table 3.3 Distribution of RVFV IgG seropositive Goats from Laikipia

Sex	Number Seropositive (%)	Number sampled
Male	5 (5)	100
Female	30 (31.6)	95

Table 3.4 Number of RVF IgG seroconversions in camels from Mpala ranch from March 2019 and April 2019.

Location	Species	Number seropositive	Number sampled	Year	Month
Mpala	Camels	13 (34.2)	38	2019	March
Mpala	Camels	3 (11.1)	27†	2019	April

† Only includes camels negative in the March survey (i.e. those seropositive or vaccinated and not seropositive prior to sampling in the March survey were removed).

3.3.2. Longitudinal Study

As explained in 3.2.1 camels from Mpala Research Centre were sampled in March and April 2019 due to worries about vaccine virus shedding after a number of camels within the herd were vaccinated with the Smithburn live attenuated vaccine. During this time three camels did seroconvert (Table 3.4). For the camels that were seropositive from the Mpala Ranch in March 2019, males (8/14, 57.1%) were more at risk compared to females (5/24, 20.8%), ($p=0.07$).

3.4. Conclusions

The purpose of this study was to determine RVF seroprevalence in livestock species in three counties in Kenya during an interepidemic period. All animals sampled in Machakos and Laikipia were born after the last major outbreak in the country (2007), therefore samples that were confirmed seropositive were identified as transmission events in the absence of formal reported cases.

Very low seroprevalence of anti-RVFP antibodies was identified in livestock species in the Kisumu county. This is supported by published risk maps and seroprevalence studies conducted in humans (Cook et al 2017). It has previously been hypothesised that the climatic conditions of western Kenya may not be conducive to RVFP maintenance, specifically high RVF seroprevalence can be correlated with seasonal flooding in semi-arid areas, whereas semi-humid areas would require extensive rainfall (LaBeaud et al 2007, Cook et al 2017).

Wildlife species may play an important role in maintenance, persistence and transmission of RVFP although the role of wildlife species as reservoir hosts is yet to be established (Rostal et al 2017). Longitudinal studies have shown long-term undetected RVFP circulation in buffalo (Beechler et al 2015). Livestock sampled in Machakos and Laikipia differed from

Kisumu in the fact that abundant wildlife species were present, and livestock could have potentially had direct interactions with these animals that may have had active infections.

As Métras et al. (2017) theorise, emergence is due to three variables: increase in vector density, availability of susceptible animals and presence of virus. It is not unreasonable to predict outbreaks may occur in the Kisumu county in the future. Like Kisumu, Mayotte is classed as a tropical region and both have favourable habitats for the RVFV mosquito vectors. RVF outbreaks in both animals and humans have recently been reported (November 2018- May 2019) on the Mayotte island (WHO 2019). This and the previous 2006 – 2007 outbreak have been linked to illegal animal movements (Métras et al. 2017, WHO 2019). With a suitable vector-livestock ratio to sustain viral persistence (Manore and Beechler, 2016), the presence of susceptible animals within Kisumu and animals that may be actively infected moving into the county from other at-risk counties, outbreaks could be reported in the future.

The variation in seroprevalence results by species from Machakos is surprising especially as RVFV outbreaks have never been reported on the Kapiti ranch. Evidence suggests that cattle have a less severe, and often, subclinical infection with RVFV than other livestock species (Davies 2003, Lichoti 2014, Fafetine 2013, Lagerqvist et al, 2013, Bird et al. 2008). This could explain how some cattle were RVF seropositive. These seroprevalence results are also within the range of seroprevalence results identified in interepidemic periods for cattle in Chapter 2. The Kapiti ranch is at a slightly higher elevation (1650-1900m above sea level (ILRI 2017)) to other areas in the Machakos county and survival of RVFV vectors and, hence, viral persistence on the farm is less likely. Although the majority of the cattle are born on the ranch there is also the possibility the seropositive cattle may have been bought from other ranches elsewhere in Kenya which could also explain the seropositivity.

The highest recorded seroprevalence from this study was documented in a herd of goats (17.9%) on a farm in Laikipia sampled in 2018. Small ruminants are known to be the most susceptible out of the livestock species. With a significant percentage of the herd seropositive, clarification through personal communications with the farmer confirmed vaccinations for RVFV had not been given to these animals. Vaccination records are valuable in terms of determining which animals should be protected and generally how efficacious vaccines are.

The longitudinal study provides evidence of seroconversion in camels from 2018 to 2019. Due to incomplete animal records, specific camels cannot be linked longitudinally between 2018 and 2019 from these farms in Laikipia, although it is known that many of the camels tested in 2018 were also tested in 2019. The longitudinal study between March and April 2019 did follow specific camels and further seroconversion was seen between these months in these animals. Between the 2018 and 2019 sampling in Laikipia and Machakos RVFV livestock and human outbreaks were recorded in several counties in Kenya including Wajir (June 2018), Garissa, Kajiado, Kitui, Marsabit, Tana River (WHO, 2018) and in (4/160) cattle in Kihingo, Murang'a. Further outbreaks occurred in late January 2019 in 4/160 cattle in Kihingo, Murang'A and in 1/140 sheep and 4/70 cattle in Shamata in late February 2019 (OIE, 2019). The farm manager at Mpala Research Centre, where camels were sampled, reported abortions in five camels during March-August 2018, which were subsequently found to be IgG positive to RVFV in a separate confirmatory diagnostic study conducted (Hewitt, D. personal communication Sept 2018). Consequently, seropositive samples were recorded in the following 2019 sampling.

The seroconversions reported in camels on the Mpala ranch during the longitudinal study between March and April 2019 were a concern, as this suggests that camels vaccinated for the pilot Smithburn vaccine trial may have shed vaccine virus. However, ELISAs results

from the vaccine field trial reported in Chapter 5 demonstrate that vaccine virus shedding is unlikely and that these seroconversions are more probably linked to a silent RVF outbreak. The outbreaks reported in January and February 2019 were approximately 170km from the Mpala ranch. These results from 2018 and 2019 in Laikipia suggest that RVFV is endemic and is circulating undetected in the area.

It should be borne in mind that these RVFV seroprevalence results may have limited generalisability, having been derived from a small number of farms from different counties. Nor can these results provide information and analyses on seroprevalence in other Kenyan counties. Even on a county level, it is expected there will be variability in seroprevalence due to other factors such as farm management, contact with wildlife and vectors, age of animals, species, climate and local environment. These samples were taken opportunistically from surveys originally undertaken for other reasons and thus the study designs were not based on specific seroprevalence studies. Some data was not available to analyse. Importantly, some camels on the farms in Laikipia did not have any unique identification, hence it was not possible to report true seroconversion in herds between 2018 and 2019 and records of age were limited.

These surveys have accounted for transparency and reported on study design and analysis following the STROBE statement (Von Elm 2007, Clark M 2018). RVFV IgG antibodies are believed to be long-lasting (Lwande et al 2015) and, unfortunately, the IgG ELISAs did not provide evidence of when seroconversion actually occurred. The study in Laikipia had multiple testing time points on the same camels, consequently, seroconversion can be demonstrated to occur within the time period between subsequent time sampling. To identify recent exposure PCR or IgM ELISAs could be used, these antibodies often peak 2-4 weeks post exposure (Niklasson et al. 1984). There is therefore a short time span in which these

antibodies can be identified in samples and due to cost and feasibility this study did not test for IgM.

As recommended in Chapter 2, to understand the transmission dynamics in context seroprevalence studies should consider concurrently testing seroprevalence in wildlife and humans as well as livestock. Integrated surveillance such as the surveillance systems used in the Kisumu county are a model platform for assessing seroprevalence of RVFV and other zoonotic diseases in livestock and humans accounting for direct contact. The syndromic surveillance platform used for veterinary purposes is also a useful surveillance system specifically for diagnosing RVFV due to its non-specific symptoms and would support surveillance in terms of disease preparedness for public health. To maximise the use of these systems, and understand life histories of animals, and hence seroconversion results, a formal animal identification system including histories of vaccination and movement of individual animals is warranted. However, these systems still rely on active reporting and assessing true seroprevalence during IEPs would need longitudinal studies. A cost-effective approach outlined by Lichoti et al (2014) is to follow up animals as a herd, setting out a specific protocol which samples herds at a specific age or known RVFV vaccination histories.

This study confirms that RVFV seropositive animals are identified in IEPs. The type of species and location of these animals are likely to be risk factors for being RVFV seropositive. Although the generalisability of the results is limited, the results corresponded with the published RVFV risk maps. Nevertheless, surveillance should still continue in counties that are deemed 'low risk' particularly as we continue to see changes in climate, environment and movement of animals and people.

4. Immunogenicity of a Chimpanzee Adenovirus Viral Vectored Rift Valley fever vaccine

4.1. Background

Simian adenovirus vector vaccines are an attractive vaccine platform particularly for outbreak potential diseases for both veterinary and medical use due to their excellent immunogenicity and safety profile (Ewer et al. 2017, Baron 2018). However, anti-vector immunity is thought to be a key factor influencing performance of adenovirus-vectored vaccines in humans but has yet to be studied in livestock.

This chapter tests anti-vector immunogenicity in four livestock species (sheep, goats, cattle and camels) to understand what titres of pre-existing antibodies to ChAdOx1 virus there are and if this correlates with RVF antigen immunity.

Viral vector vaccines, in general, are able to circumvent many issues which are reported with conventional vaccines such as those that are live attenuated. However, a large issue for viral vectored vaccine use reported in humans (particularly human adenoviral vectored vaccines) is pre-existing immunity to the viral vector which may inhibit the ability of the vaccine to elicit protective neutralising antibody responses (Buchbinder et al. 2008, McElrath et al. 2008). This issue can be overcome by administering higher doses or giving the vaccine in a prime-boost regime (Ewer et al. 2016).

Pre-existing immunity to viral vectors can result in antibody-mediated neutralisation of the vaccine vector, restricting the viral vector delivery to cells and therefore reducing the immunogenicity and efficacy of the vaccine. It is understood that neutralising antibodies against the adenovirus target the adenovirus surface loops (hypervariable regions) of the hexon. This mechanism seems to play the most dominant role in anti-vector immunity (Fausther-Bovendo and Kobinger 2014). Neutralising antibodies can also be elicited against

the penton base, fiber and core (Wohlfart 1988, Toogood et al. 1992, Fausther-Bovendo and Kobinger 2014).

To avoid the issue of pre-existing immunity to human adenoviral vectored vaccine, simian adenovirus viral vector vaccines have been developed as little or no ChAd seropositivity in human populations has previously been reported (Dudareva 2009, Dicks 2012, Nebie 2014, Jian et al. 2014, Ewer 2017). Although the emphasis of this thesis is on the application of the veterinary ChAdOx1 RVF vaccine, the definitive aim of the research programme is to create a One Health vaccine that can be used in both humans and livestock species, hence the rationale for using the simian adenovirus. ChAds are versatile as they are able to induce humoral and cellular immunity against a range of pathogens (Antrobus 2012, Swadling et al. 2014, Bliss et al. 2017). Research has evaluated natural exposure of chimpanzee adenoviruses (ChAds) in mice and humans (Dicks 2012, Dudareva 2008) but anti-vector immunogenicity in livestock species has not been investigated. Specifically, ChAdOx1 has been tested in livestock species: a ChAdOx1 vaccine encoding mycobacterial antigen 85A has been tested in cattle and vaccine efficacy and safety trials for ChAdOx1 RVF vaccine have been tested in sheep, goats, cattle and camels but viral vector immunogenicity for these species has not been assessed (Dicks, Guzman 2015, Warimwe 2016, Stedman 2019).

4.2. Methods

The samples used for this study came from two separate programmes of work. ChAdOx1 RVF (formally ChAdOx1 GnGc) vaccine studies were conducted in Kenya, Saudi Arabia and the UK (Warimwe et al. 2016) and a ChAdOx1 RVF dose titration study was conducted in the UK. All animal experiments were reviewed and approved by the International

Livestock Research Institute's Institutional Animal Care and Use Committee (ILRI-IACUC) as well as The Pirbright Institute Animal Welfare & Ethical Review Board (AWERB).

4.2.1. Vaccine efficacy studies

The study design of these trials has already been published elsewhere (Warimwe 2016). Briefly, Dorper sheep, Galla goats and Holstein-Friesian cattle were vaccinated with 10^9 infectious units of ChAdOx1 RVF vaccine in Kenya. Animals were challenged at four weeks post-vaccination with 10^7 plaque forming units of heterologous virulent RVF strain 56/74IN. Serum was taken at Day 0 pre-vaccination and Day 28 post-vaccination. In parallel the vaccine was also evaluated in immunogenicity studies in Merino sheep and Holstein-Friesian cattle in the UK and dromedary camels in Saudi Arabia.

4.2.2. Dose escalation study

This study was conducted at The Pirbright Institute in the UK. Three doses of freeze-dried formulation of the ChAdOx1 RVF were used: 10^6 , 10^7 , 10^8 infectious units (iu). The study was conducted in Mules sheep (n=15) and Holstein-Freisen cattle (n=21). Sample size calculations were conducted, and five sheep and seven cattle were recruited per group. Animals were vaccinated intramuscularly on the right base of the neck with 2ml of vaccine. Samples were taken pre-vaccination at day-1 and then day 1, 3, 5, 7, 14 and at day 21 post vaccination.

4.2.3. Anti-vector neutralising antibody assay

A secreted embryonic alkaline phosphatase-reporter (SEAP) assay was used to measure neutralising antibody titres against ChAdOx1. This method has previously been described in Dicks et al. (2012). SEAP is a reporter gene that can be inserted into the ChAdOx1 viral vector backbones and is used to assess anti-vector antibody neutralisation titre of the livestock serum samples. Briefly, GripTite 293 Macrophage Scavenger receptor (MSR)

cells (Invitrogen cat no. R795-07) were seeded at 3×10^4 cells/well. Livestock serum samples were heat inactivated at 56°C for 2 hours before use. ChAdOx1 (1041 stock diluted in 1:100 dilution with Phosphate Buffer Saline (PBS)) and the heat inactivated sera were pre-incubated for 1 hour at 37°C. Serum dilutions (in 0% FBS phenol red free DMEM - Life Technologies cat no. 31053028) of 1:18, 1:72, 1:288, 1:1152 and 1:4608 were used. A positive sample with known neutralising ChAdOx1 titres as well as a known unreactive serum were used for positive and negative controls respectively (both samples came from Flu005 clinical trial at The Jenner Institute). The serum-virus mixture was transferred to the GripTite cells and left for 1 hour and then removed and replaced with 10% FBS phenol red free DMEM for 24 hours. Cell supernatants were then extracted and used to measure SEAP concentrations using the Tropix PhosphaLite Chemiluminescent Assay Kit (Tropix PhosphaLite Chemiluminescent Assay Kit, Applied Biosystems UK). To assess this a Varioskan Flash luminometer (Thermo-Fisher) was used to measure luminescence. Antibody titres were measured as the dilution of serum at which the secreted alkaline phosphatase in permissive cells falls to 50% of the expression in cells infected in the absence of immune serum.

4.2.4. RVFV Gn ELISA

My colleague Daniel Wright completed the RVFV Gn ELISAs at The Jenner Institute, University of Oxford. Briefly, plates were coated with 1µg/ml Gn monoclonal antibodies (4D4), 50µL per well, and left at room temperature overnight covered in cling film and foil. The next day plates were washed with PBS/0.05% tween six times. Plates were blocked with 100µL of blocking solution (Casein) and left for an hour at room temperature. The blocking solution was flicked off and 2µg/ml of Gn diluted in casein was added to the 4D4 coated plates. This was left for 1 hour at room temperature. Dilutions of the serum with casein were made in deep well plates at 1:100 concentration. Plates were washed with PBS and tween

six times again. The pre-diluted serum was transferred to the corresponding wells on the coated plate, 50µl in each plate and incubated for 2 hours at room temperature. Plates were washed with PBS and tween solution. HRP-conjugated antibody diluted in casein (1:500 cattle, 1:15,000 sheep/goat) was added to the wells, 50µl to each well. This was incubated for 1 hour at room temperature. Plates were washed again six times with blocking solution. The TMB substrate was added 100µl to each well. The plates were incubated in the dark (covered in foil) at room temperature for approximately 45 minutes after which 100µl of the stop solution was added to each well. Plates were then read at 450nm on BioTech Microplate Reader and antibody units were calculated.

4.2.5. Statistical analyses

The Shapiro-Wilk test was used to test the data distribution for normality. The Spearman-Kärber method was used to calculate the neutralising titres against the Rift Valley fever virus. Non-parametric tests were conducted using R statistical software environment (version 3.3.3) (R Core Team 2013). A Wilcoxon test was used to assess significance of increasing neutralising antibodies titres from Day 0 pre-vaccination to Day 28 post-vaccination. The Wilcoxon test for paired data was also used for establishing significance of increasing neutralisation antibodies from Day 0 pre-vaccination to Day 21 post-vaccination in the dose study. Linear regression was used to measure correlation between anti-ChAdOx1 neutralising antibodies and RVFV Gn antibodies.

4.3. Results

4.3.1. Livestock animals show pre-existing antibodies to the ChAdOx1 vector

Pre-existing anti-ChAdOx1 antibodies were identified in all four livestock species tested (n=88) (Figure 4.1). Goats elicited the highest pre-existing antibodies to ChAdOx1 with camels being lower than any other species. Antibodies were significantly higher in Kenyan

cattle than UK cattle ($P=0.028$), conversely UK sheep had significantly higher antibodies than Kenyan sheep ($P=0.0001$). Neutralising antibody titres that were $<1:18$ were not within the limits of the assay detection and were therefore considered negative.

4.3.2. Measuring anti-vector antibodies in vaccinated livestock

Anti-ChAdOx1 neutralising antibodies significantly increased post-ChAdOx1 RVF vaccination in sheep ($p=0.001$), goats ($p=0.001$) and cattle ($p=0.0002$) at day 28-post vaccination. Contrary, boosting of neutralising antibodies was not stimulated in dromedary camels (Figure 4.2).

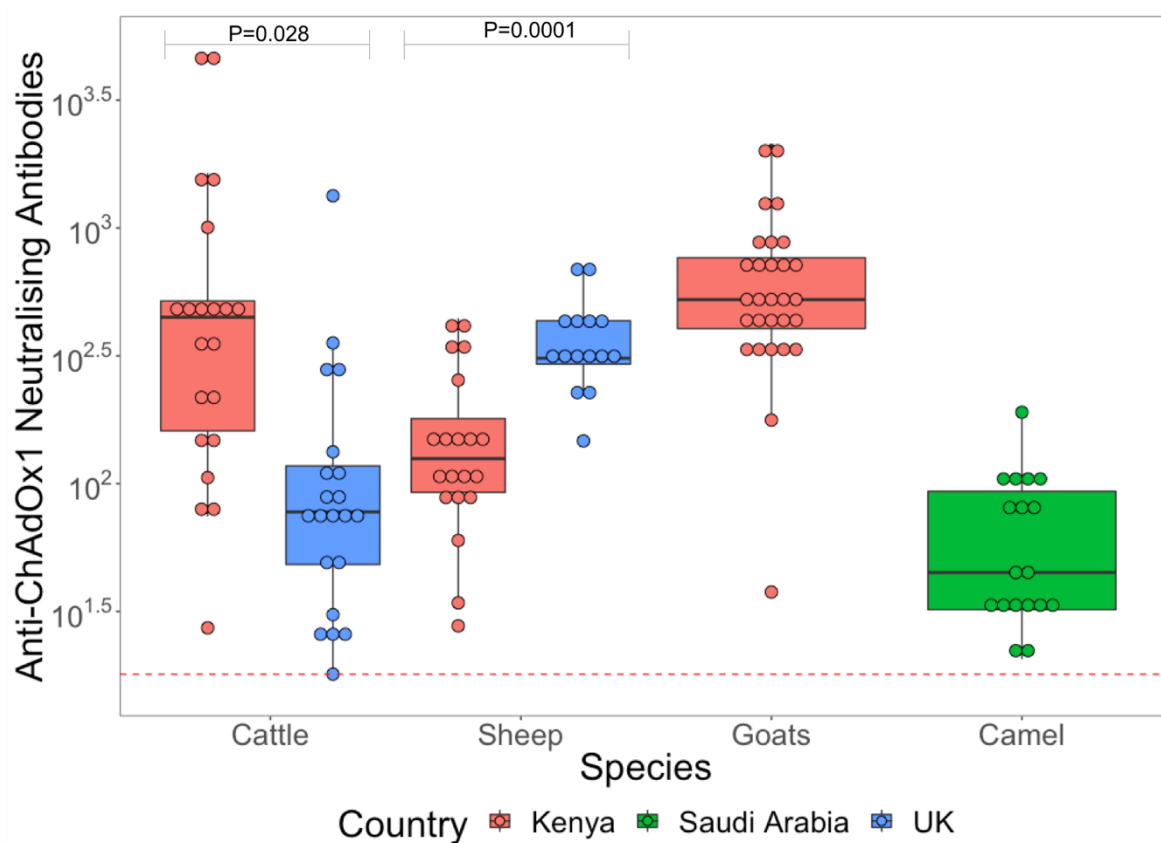


Figure 4.1 Livestock species from different countries have pre-existing antibodies to ChAdOx1 virus (Cattle=21, Sheep=20, Goats = 29, Camels=18). The dotted red line represents the lower limit of detection for the neutralisation assay. Bars in boxplot represent median and IQRs values of neutralisation antibody titres against ChAdOx1 reported in the different species. Dots represent individual animals.

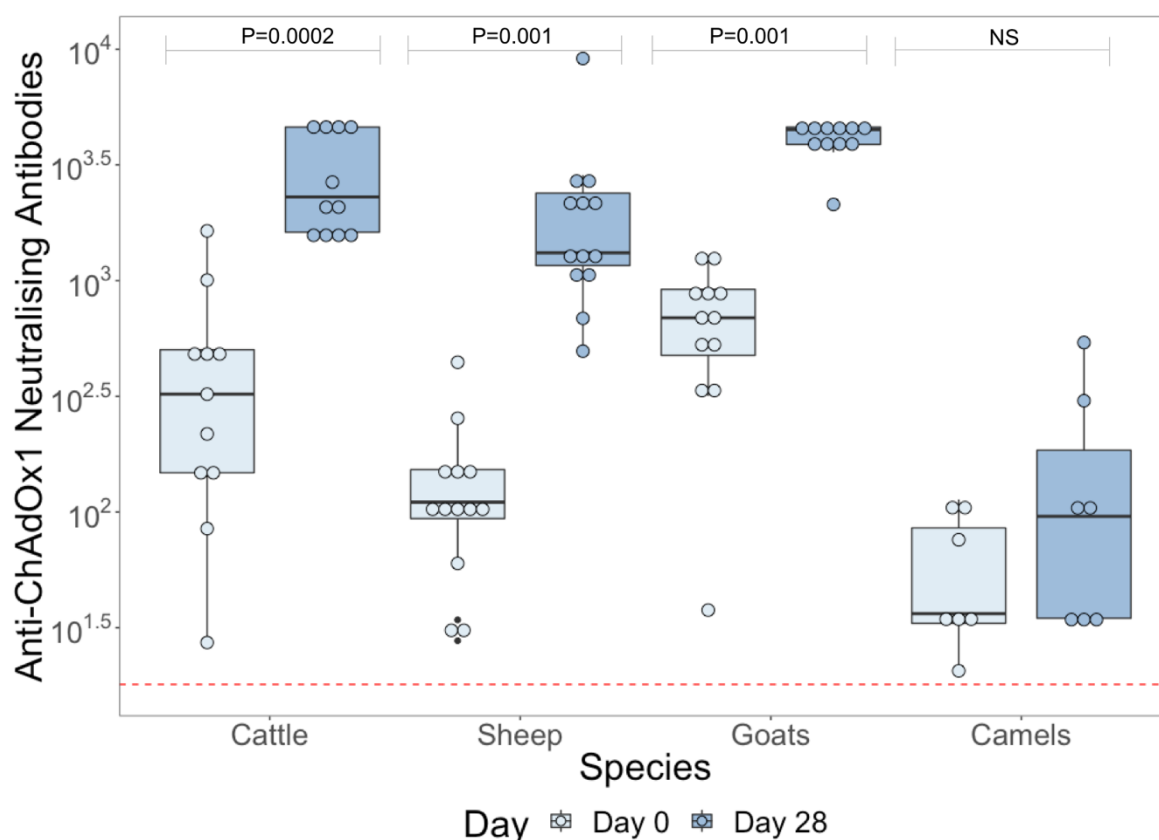


Figure 4.2 Anti-ChAdOx1 neutralising antibodies significantly increase after vaccination with ChAdOx1 vaccination (Day 0 vs. Day 28). The dotted red line represents the lower limit of detection for the neutralisation assay. Bars in boxplot represent median and IQRs values of neutralisation antibody titres against ChAdOx1 reported in the different species. Dots represent individual animals. (Cattle=21, Sheep=20, Goats=29, Camels =18)

4.3.3. Assessing correlation of anti-vector antibodies and RVFV Gn antibodies in vaccinated livestock

No significant correlation was found between neutralising antibody responses to ChAdOx1 (Viral vector) at day 0 and antibodies to the RVF Gn protein at Day 28 in sheep ($p=0.3879$, $r^2=0.06844$) (Figure 4.3), cattle ($p=0.6731$, $r^2=0.021$) (Figure 4.4) and goats ($p=0.01294$, $r^2=0.5147$) (Figure 4.5). Unfortunately, RVF Gn ELISA results were not available for the camels.

4.3.4. Assessing effect of vaccine dose to anti-vector antibody response

The effect of ChAdOx1 RVF dose was assessed on neutralising antibody titres to ChAdOx1. Neutralising antibody titres significantly increased as dose increased in cattle (Figure 4.6) however there was no significant increase in neutralising antibody titres as dose increased in sheep (Figure 4.7).

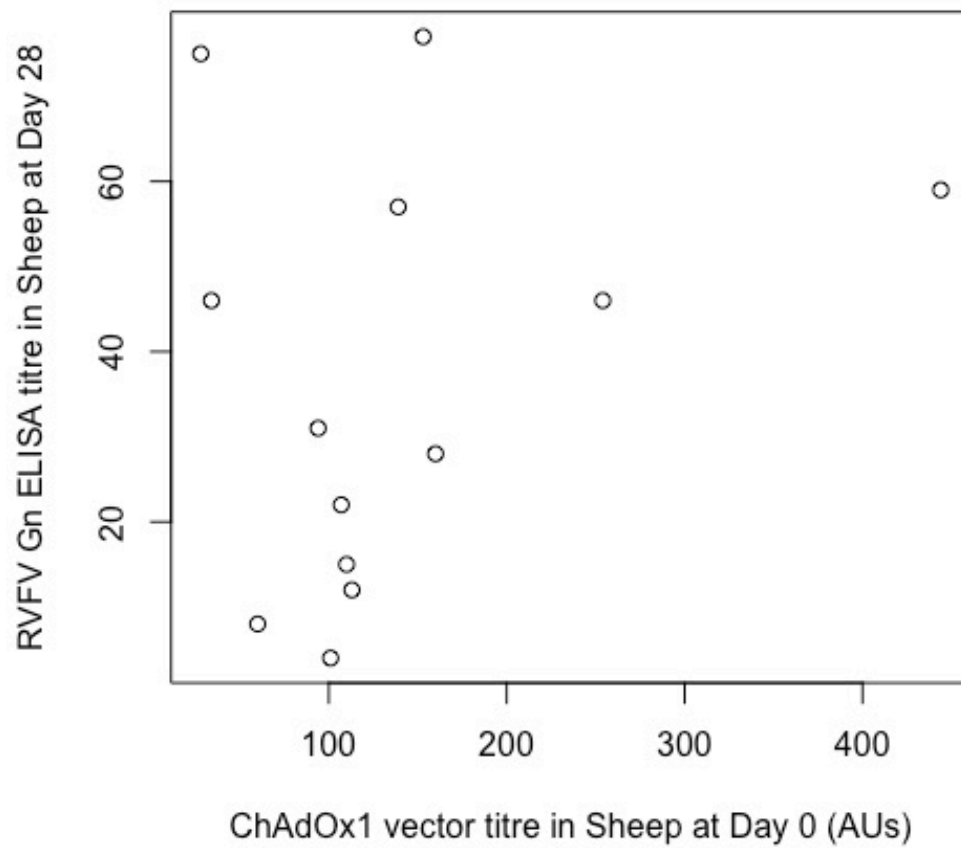


Figure 4.3 There is no correlation between RVFV Gn ELISA titre and the log of anti-ChAdOx1 neutralising antibodies in sheep.

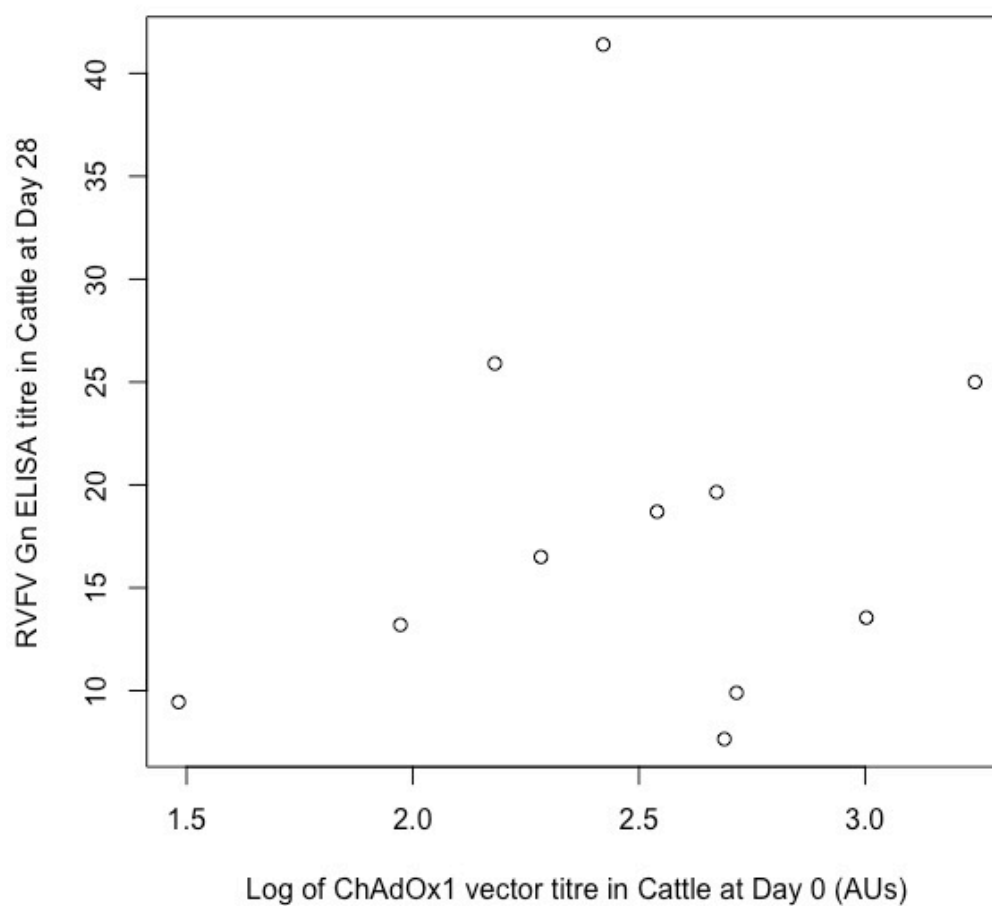


Figure 4.4 There is no correlation between RVFV Gn ELISA titre and the log of anti-ChAdOx1 neutralising antibodies in cattle.

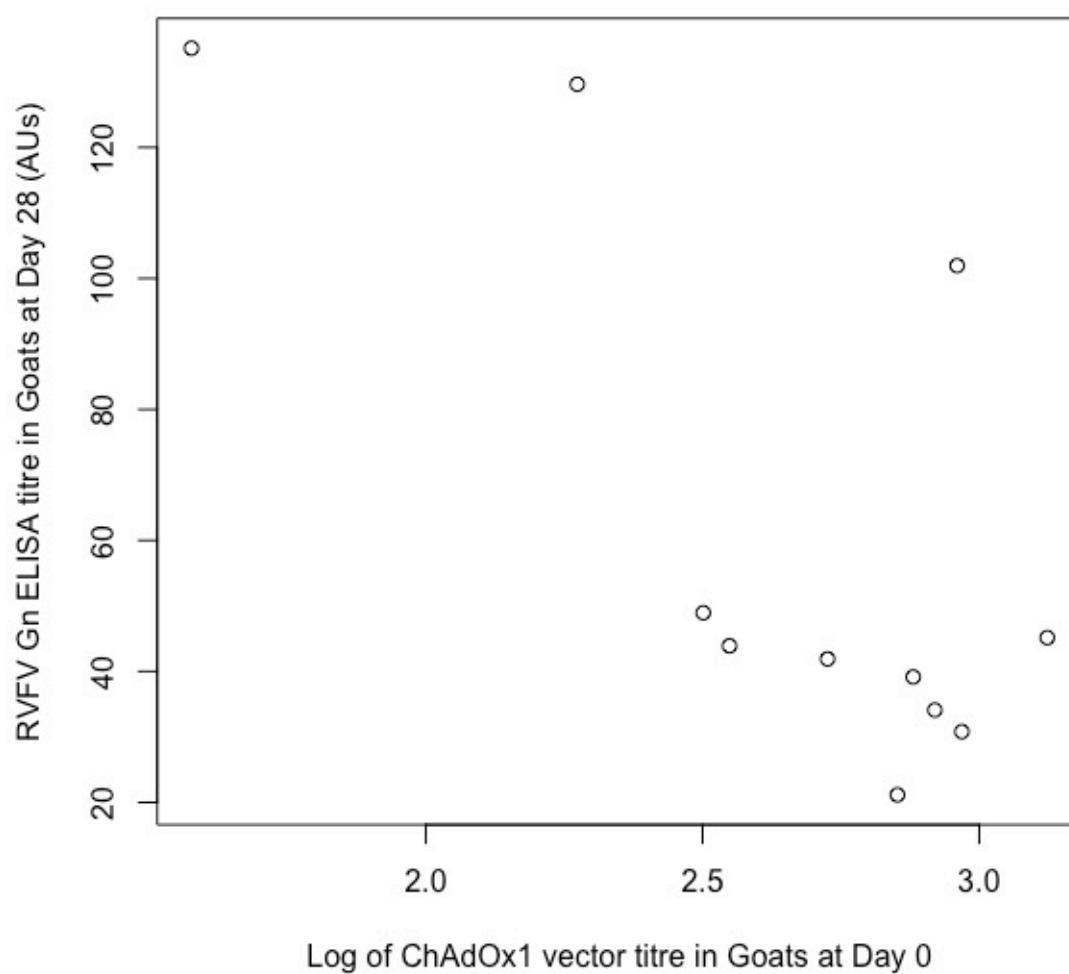


Figure 4.5 There is no correlation between RVFV Gn ELISA titre and the log of anti-ChAdOx1 neutralising antibodies in goats.

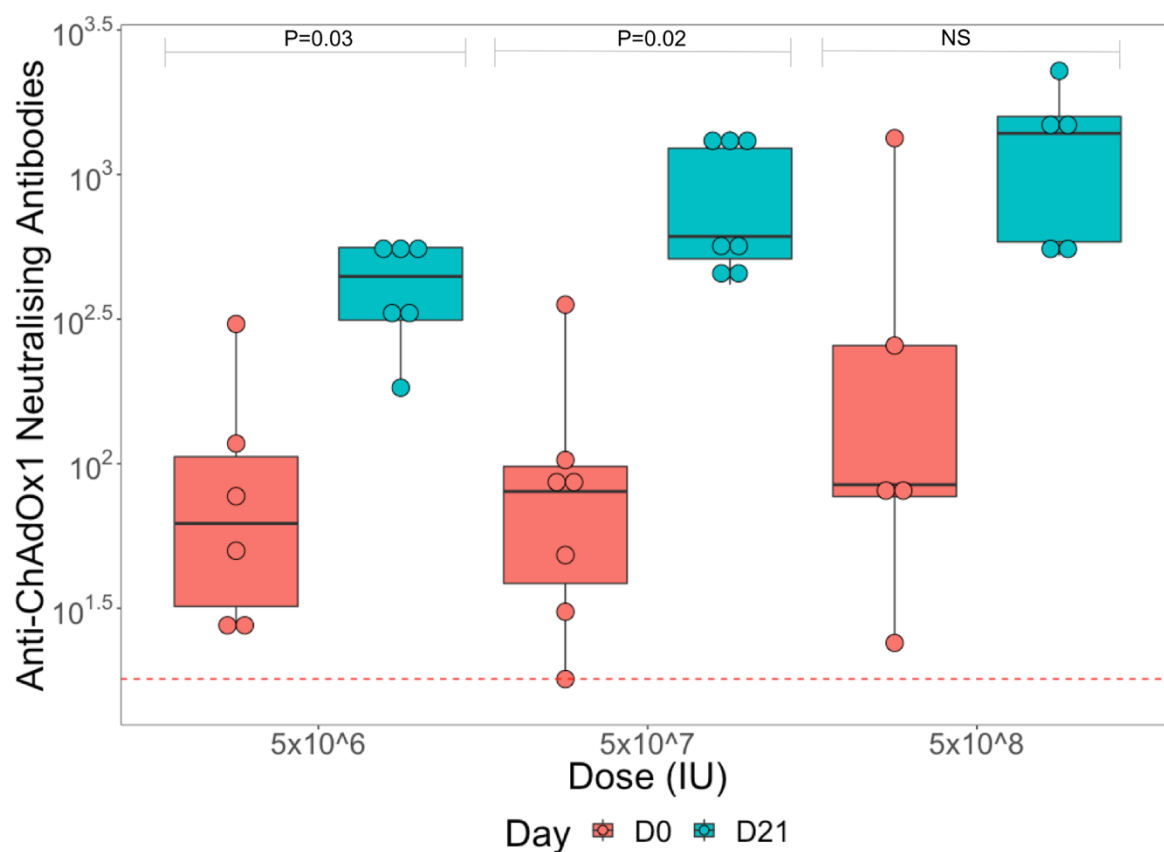


Figure 4.6 Anti-vector antibody responses comparison with dose of vaccine in cattle (n=21). The dotted red line represents the lower limit of detection for the neutralisation assay. Bars in boxplot represent median and IQRs values of neutralisation antibody titres against ChAdOx1. Dots represent individual animals.

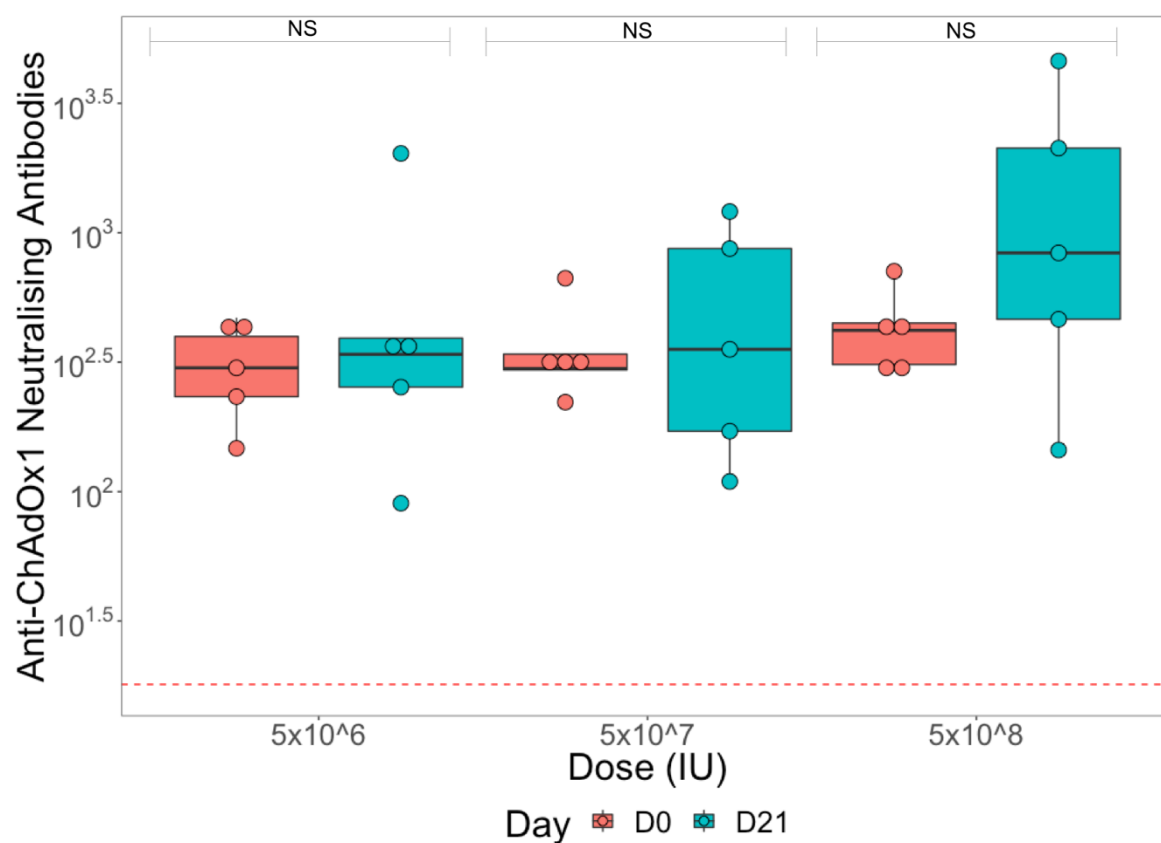


Figure 4.7 Anti-vector antibody responses comparison with dose of vaccine in sheep (n=15).

The dotted red line represents the lower limit of detection for the neutralisation assay. Bars in boxplot represent median and IQRs values of neutralisation antibody titres against ChAdOx1. Dots represent individual animals.

4.4. Conclusions

This study has demonstrated that livestock species do have pre-existing neutralising antibodies to ChAdOx1 at varying magnitudes. Antibodies to the ChAdOx1 viral vector assessed by ELISA have been reported in cattle (Dicks 2015). A study testing a prime-boost vaccine regime for ChAdOx1 MERS in Rhesus macaques found that even though antibodies to ChAdOx1 were present before the second vaccination, this did not prevent boosting of neutralising antibodies when vaccinated with a ChAdOx1 MERs vaccine (Van Doremalen, submitted).

Pre-existing neutralising antibodies elicited in camels were substantially lower than other livestock species. This could be related to distinct immunological mechanisms in camels or absence of natural exposure to the Y25 virus in Saudi Arabia where these camels were sampled. The magnitude between and within species and countries varied significantly.

A substantial amount of research has assessed immunogenicity of viral vectors in human populations. In human clinical studies, antibody neutralisation titres between 1:18-200 are considered low (Folegatti 2019, Antrobus 2014, McElrath 2008, Nébîé 2014). Pre-existing antibodies in cattle, sheep and goats identified in this study are overall higher than this suggesting the considered range for low neutralisation titres in livestock species may be higher than that of humans. Future studies should specifically assess neutralising antibody responses in young animals who will not have been exposed to the viral vector vaccine and thus provide a more suitable threshold.

The nature of the assay means the results generated are based on percentage of virus only cells which are then interpolated to determine dilutions required to reduce the SEAP concentration by 50%. This means results are relative to the virus only rather than an

absolute value. The assay is suitable for seroprevalence analysis but to understand the mechanisms behind ChAd immunity would require other immunological testing targeting cellular (ChAd specific T cells) or parts of the innate response (type I activated NK cells) (Fausther-Bovendo and Kobinger 2014). As a seroprevalence test it is a relatively quick and easy assay. However, if field trial studies were to be conducted with larger sample sizes and multiple time points in resource-limited settings, optimising a peptide ELISA based on conserved regions of the ChAdOx1 genome would be worthwhile.

ChAdOx1 and other simian adenoviruses are phylogenetically grouped within human adenoviruses species; ChAdOx1 is in species E. Although closely related, the hypervariable regions of the hexon are significantly different from other adenoviruses including human adenovirus 5 (Colloca 2016). It is questioned how these animals could have been exposed to a chimpanzee virus. Multiple studies have demonstrated that chimpanzee adenoviruses do not cross react, specifically ChAdOx1 does not cross react with ChAd63 (Dicks 2012, Folegatti 2019) however it could be speculated that ChAdOx1 (Y25) may cross-react with a livestock-specific adenovirus for example a bovine adenovirus. This may explain why a high proportion of animals had pre-existing neutralising antibodies (Dudareva 2008). Associations in sequence homology between bovine and human adenoviruses have previously been identified (Hu 1983). Future research should implement bioinformatic research to identify homology between livestock species-specific adenoviruses and ChAds.

The samples used in this study were taken from previous laboratory studies. This research warrants assessment with larger sample sizes and in the context of the field. Field trial studies in areas where RVFV is a risk would measure responses in target populations for RVFV vaccination. If anti-vector immunity were to have an effect on response to the vaccine antigen there are alternative strategies that could be utilised or combined with others to aid

in vaccine protection despite vector immunity. Prime-boost strategies have been implemented for other vector vaccine trials. This provides sufficient immune responses for protection using alternative routes of immunisation (delivery can bypass anti-vector immunity) and trialling other adenovirus serotypes (Fausther-Bovendo and Kobinger 2014).

Despite pre-existing antibody titres in all livestock species, a significant boosting effect of the neutralising antibodies after vaccination was recorded in cattle, sheep and goats and this had no effect on the ChAdOx1 RVF vaccine efficacy. Similar results have been recorded with the ChAd63 ME-TRAP vaccine: anti-vector neutralising antibodies were not correlated with antibody or T cell responses to the vaccine antigen (Bliss 2017). Antibody titres elicited post-vaccination were comparable to those elicited in humans when vaccinated with a ChAdOx2 vaccine against HAV (Folegatti 2019) and a ChAd63 vaccine for malaria (Bliss 2017) although it is unknown how comparable this is between species. Neutralising antibody titres did not boost after vaccination in camels although neutralising antibody responses to RVF were elicited (Warimwe 2016). This compounds the need to conduct further work into the anti-vector immunological responses of camels to understand these mechanisms more closely. This chapter has not assessed the T cell response to ChAdOx1, but these may also play a role in anti-vector immunity (Bliss 2018) and thus require further research. Assessing ChAd specific T cells would provide a complete understanding of ChAdOx1 pre-existing immunity.

Numerous ChAd vaccines are currently in development for outbreak pathogens (Ewer 2017) and those of medical importance, due to their attractive features including good safety profiles, induction of potent cellular and humoral immunity, circumventing pre-existing immunity in humans and a good vaccine platform for stockpiling. In the veterinary field, they also pose additional attractive characteristics, most notably no issues with reversion to

virulence in outbreak settings and enable differentiation between infected and vaccinated animals when used in conjunction with diagnostic tests.

This study has shown neutralising antibodies to ChAdOx1 are mounted in livestock species prior to being vaccinated, whether this is natural exposure to the originating Y25 virus or there is a level of cross-reactivity will need to be examined further. In any case ChAdOx1 RVF vaccine efficacy is not impeded. This study is the first to assess neutralising antibody responses to a viral vector vaccine across multiple veterinary species. The findings indicate that although animals are exposed to ChAdOx1, these viral-vector viruses are successful platforms for veterinary use.

5. A non-inferiority Rift Valley fever vaccine field trial to assess vaccine immunogenicity in multiple livestock species in Kenya: Study design and regulatory perspective

5.1. Background

5.1.1. Rationale for non-inferiority field trial in multiple species

This chapter outlines operational aspects of designing and implementing a vaccine field trial in multiple livestock species for an emerging zoonotic disease. The overarching goal is to develop a ‘One Health vaccine’ for both veterinary and human use. The chapter reflects on the regulatory and logistical processes required to set-up a non-inferiority vaccine field trial in multiple livestock species. There were multiple aims in conducting this trial:

1. As RVFV is an outbreak pathogen it is difficult to reliably identify high incidence areas for vaccine efficacy trials that assess protection. This confined non-inferiority field trial works as a bridging field trial between small scale laboratory challenge experiments and large multi-farm field trials. The trial assesses immunogenicity in indigenous livestock species under field conditions.
2. The study was run as a randomised controlled blinded non-inferiority trial. The experimental aim was to directly compare a novel vaccine to a commercialised one. If the non-inferiority criteria are met the novel vaccine is eligible to be registered and licensed in the country.
3. The veterinary vaccine field trial was run to the principles of veterinary good clinical practice (vGCP) and externally monitored by appropriately qualified staff. This was implemented to enhance integrity and credibility. Monitoring records can be used as supporting information for regulatory bodies, included in dossiers for veterinary vaccine licensure and for informing decisions to undertake further clinical trials.

As mentioned in the introduction (Chapter 1) there are veterinary vaccines for RVFV commercialised in multiple countries, although these vaccines are suboptimal: the live attenuated vaccines cannot be used in outbreak situations due to the worries about reversion to virulence and reassortment with the wildtype virus and in pregnant animals they can cause

abortions; the killed vaccine is poorly immunogenic and diagnostic tests are unable to differentiate between infected and vaccinated animals. There is also no commercialised human vaccine for the disease. The development of the ChAdOx1 RVF vaccine has already shown it is able to circumvent some of these issues: it encodes the RVF Gn and Gc glycoproteins only (so is DIVA compatible) and it is safe to use in pregnant sheep and goats (Stedman et al. 2019).

A non-inferiority study design is different to a superiority or equivalence study. Whereas a superiority trial aims to demonstrate that a new vaccine is better than the existing treatments and an equivalence trial aims to show that there is no difference between the two vaccines, a non-inferiority trial aims to show that the novel vaccine is not substantially worse than the commercialised product (Hahn 2012). Freise et al. (2013) outline four distinct components needed for a non-inferiority trial:

1. The hypotheses (null and alternative) need to be switched from what is used for superiority tests: the alternative hypothesis is that there is no difference between treatment groups. The main interest is showing the difference in treatments is not too much different from zero and hence showing that the test treatment is at least as effective as the control (non-inferior).
2. Selecting an appropriate control
3. A suitable non-inferiority margin and investigational drug needs to be determined; this needs to be selected prior to commencing the trial. The non-inferiority margin is the maximum difference in treatment effectiveness and accounts for the degree of efficacy that could be tolerated.
4. Interpretation of the results of the study is made appropriately. This is typically achieved by calculating the two-sided 95% confidence intervals of the difference between the effects of the control and test treatment. These confidence intervals are

compared with the non-inferiority margin. If the difference favours the novel treatment and does not fall in the margin it is classed as non-inferior and superior (Figure 5.1 case 1), if it favours the novel treatment but falls within the non-inferiority margin it is non-inferior and not superior (Figure 5.1 case 2). If the confidence intervals fall within or across both sides of the margin, non-inferiority is not shown (Figure 5.1 case 3 and 4). If the difference between the effects of the control and test treatment favour the commercial vaccine and do not fall within the non-inferiority margin the test treatment is classed as inferior (Figure 5.1 case 5).

Trial assay sensitivity is an essential component for understanding the results from a non-inferiority trial. Assay sensitivity is defined as the ability to distinguish an effective treatment from a less effective/ineffective treatment. If a treatment is found to be non-inferior it could be that both treatments are similarly effective or ineffective or that the trial assay lacks sensitivity.

Although the majority of non-inferiority trials conducted are for medical purposes there are a number that have been used for veterinary clinical trials (King et al. 2018, Raferty et al. 2019). Both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) provide recommendations on undertaking non-inferiority trials for veterinary products (CVM 2012, CHMP 2006).

The non-inferiority design for this study directly evaluates the novel ChAdOx1 RVF vaccine against a vaccine that has already been commercialised (Smithburn) aiming to show the antibody response (via FRNT₈₀) generated for the ChAdOx1 RVF vaccine is not significantly worse than that generated by the commercialised vaccine (Figure 5.1). A non-inferiority design was chosen as the ChAdOx1 RVF vaccine has additional benefits as a

RVF vaccine compared to the Smithburn vaccine. It is DIVA compatible and has shown preliminary success on pregnant sheep and goats. It is a viral vector vaccine, encoding the RVF glycoproteins only and so the issues of reversion to virulence during an outbreak are minimal. These characteristics make the vaccine desirable and eligible to be commercialised even if the vaccine efficacy is lower than the Smithburn vaccine to a predefined level. This pre-defined level is the non-inferiority margin and accounts for the degree of efficacy that could be tolerated given the additional benefits. The Smithburn vaccine was chosen as the control vaccine as it is the most widely used commercial veterinary vaccine for RVF.

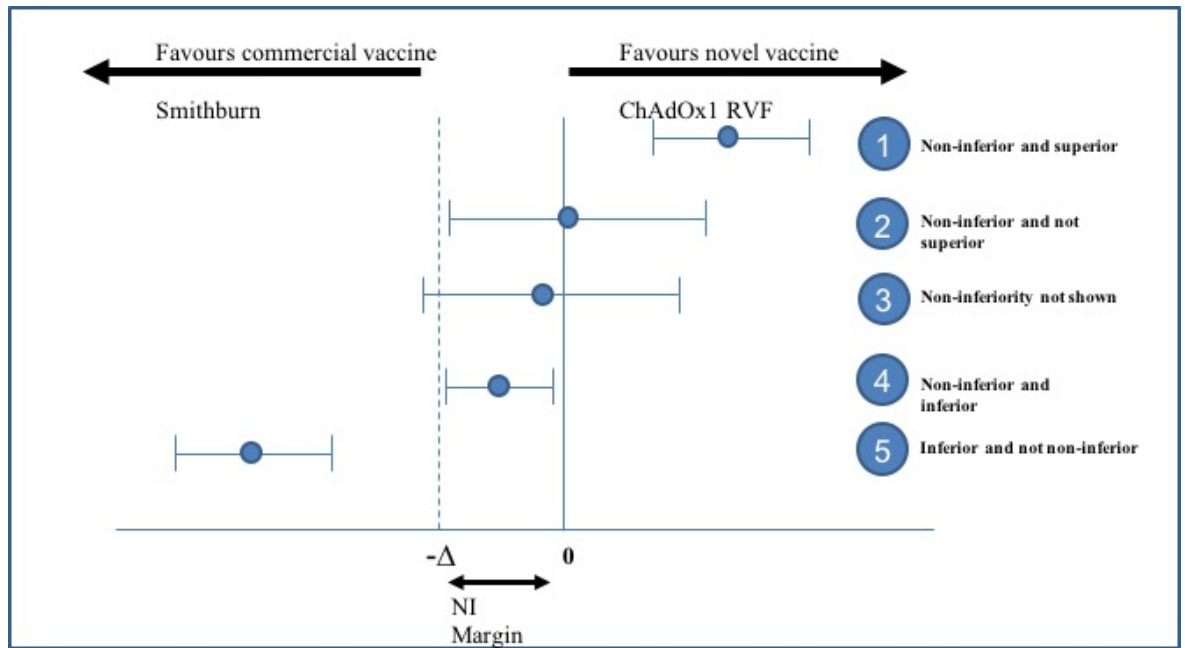


Figure 5.1 Forest plot of possible non-inferiority outcomes.

Adapted from Schumi and Wittes 2010

The variable nature of RVF outbreaks, like many other zoonotic diseases, means it is difficult to conduct a vaccine efficacy field trial that would show protection against the disease directly: although there are recognised methods to predict outbreaks, it would be challenging to be operationally ready; the frequency of infections is low; and there is an ethical consideration for personnel being exposed to the virus, as well as the local environment and other livestock and wild animals. In the absence of a vaccine efficacy trial an immunogenicity trial can be conducted to assess the immunogenicity of the vaccine.

In veterinary medicine, clinical field trials are predominantly coordinated by the private sector. Clinical trials for vaccines, and other veterinary health products are only conducted if the product is likely to be profitable. Publishing of field trial results from the private sector is limited (Knight-Jones et al. 2014). Typically, veterinary research focuses on the proof of concept research which utilises some guidelines such as the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines but does not generally follow veterinary Good Clinical Practice (vGCP). Since the most recent Ebola outbreak in West Africa (2013-2016) there has been a paradigm shift in the approach to vaccine development for epidemic diseases (Noad et al. 2019). Vaccines for these pathogens are not attractive to commercialisation and the private sector due to the limited market, the sporadic nature of outbreaks and typically occurring in resource-poor settings (both in terms of infrastructure and socio-economically). Local and inter-governmental organisations (WHO) and public-private coalitions (Coalition for Epidemic Preparedness Innovations) are directly supporting the commercialisation and investment into these vaccines, specifically for neglected tropical diseases and those that have outbreak potential which would otherwise not have been taken to market (CEPI 2017, Noad et al. 2019) and therefore research organisations are leading such field trials rather than the private sector.

5.1.2. Regulatory requirements

To conduct veterinary vaccine field trials there are numerous documents outlining what is required for regulatory purposes. The European Agency for the evaluation of Medicinal Products and International Cooperation on Harmonization of Technical Requirements of Veterinary Medicinal Products (a trilateral programme between the EU-Japan-USA) (VICH) has produced guidelines on Veterinary Good Clinical Practice (vGCP) (VICH Steering Committee 2000). These guidelines are used to inform how veterinary product clinical trials should be designed and conducted ensuring integrity and credibility. The principles of vGCP should be adopted when producing data that will be submitted to regulatory authorities. It acts as an ethical and scientific quality standard for clinical trials. These standards are routinely used in the private sector. The European Medicines Agency (EMA) has also produced guidelines on target animal safety for veterinary live and inactivated vaccines (VICH Topic GL44) which are international standards (European Medicines Agency 2008).

The European Pharmacopeia emphasises that veterinary vaccine efficacy and safety is typically demonstrated through challenging vaccinated and unvaccinated animals in laboratory experiments and should only be supplemented with data from field trials if justified (VICH Field trial with veterinary vaccines 2000). Although this fulfils regulatory requirements there are important limitations which should be considered and may have an impact on vaccine effectiveness and performance in the field (Knight-Jones et al. 2014, Lyons et al. 2019). These include: the statistical confidence of results when using small sample sizes and length of follow up (Goris et al. 2007, Knight-Jones et al. 2014), accounting for variation in environments, for example age, breed of animals, maintenance of cold chain, variable nature of the challenge virus and individual exposure to it. The study design of this trial will address some of these issues to elucidate vaccine effectiveness, how to operationally implement such a study in the field as well as non-inferiority immunogenicity.

This chapter provides guidance on the implementation, coordination and monitoring of the field trial as well as critical observations on the logistical and operational successes and challenges encountered whilst implementing a vGCP vaccine field trial for an emerging zoonotic virus. This trial was the first time a genetically-engineered vaccine was to be tested for veterinary purposes in the field in Kenya, requiring multiple approvals and communications with national government officials.

5.2. Methods

5.2.1. Study Design

The study was run as a parallel group, randomised, double blinded non-inferiority trial in four livestock species indigenous to Kenya: Red masai/Dorper cross sheep, Galla goats, Boran cattle and dromedary camels. Indigenous breeds were used as a true representation of the types of animals at risk of RVFV transmission and hence targets for vaccination. Animal breed can have an effect on susceptibility to disease. For example, the red masai sheep is considered to be relatively resistant to parasites such as *Haemonchus contortus* (Baker 2003). Breeds of animals were therefore kept the same for the experiment.

The primary endpoint was to determine the non-inferiority of ChAdOx1 RVF compared to Smithburn (Riftvax™, Kenya Veterinary Vaccines Production Institute, KEVIVAPI) based on seroconversion using neutralising antibody responses at 28 days post vaccination. Secondary endpoints included following up a subset of these animals for 12 months to assess magnitude, quality and durability of RVFV antibodies in vaccinated animals.

Animals were allocated into three groups: the investigational product, ChAdOx1 RVF vaccine group (n=80); the comparator vaccine, Smithburn (Riftvax™) vaccine group (n=80)

and a placebo (PBS only) group (n=20). The placebo group acted as a way to monitor for natural exposure to RVFV, particularly in an area that is classed as high risk for RVFV transmission on risk maps. The farm itself has never recorded a RVF outbreak (Chapter 3). Samples from the placebo group were used as controls on all experimental assays run for this thesis and the trial overall. After day 28 some animals were randomly dropped from the study so the ChAdOx1 RVF vaccine group and the Smithburn group had a total of 40 animals and the placebo group remained the same with 20 animals. This was for follow-up secondary endpoint study (not part of the thesis). The random removal of animals post-day 28 was conducted by the unblinded statistician so field trial personnel and investigators remained blinded.

For ease of handling, and to keep from unwanted pregnancies, animals were split into two herds based on sex. All animals were ear-tagged with a trial study number that individually identifies them including which treatment group they were assigned to (once unblinded). Treatment and sampling were administered over consecutive days but retained specific study day. For example, day of treatment was always Day 0. Vaccination and sampling was staggered by species so field work sampling could focus on one species at a time for the first 28 days post-vaccination. This was due to the time and work required for sampling up to the primary endpoint. Sampling started in March 2019 and continued until October 2019 for samples up to day 28. The remaining 12-month follow up study is set to finish in June 2021. Appendix A5.1 shows an example of the gantt chart.

The longitudinal nature of the field trial utilised the opportunity to take multiple sample types for the primary endpoint analysis (serum at day 28 post-vaccination for neutralising antibody assays) as well as secondary endpoint analysis: nasal and rectal swabs for PCR assessment of adenoviral shedding; whole blood for vaccine viraemia; serum and plasma

for magnitude, quality and durability of RVF antibody using ELISA and virus neutralising antibody assays; and Peripheral Blood Mononuclear Cells (PBMCs) to assess cellular responses using flow cytometry (Table 5.1). Although I coordinated the collection of secondary endpoint samples, the analysis is not included in this thesis due to the amount of work involved. These samples are currently being used as part of my colleague, Danny Wright's DPhil project.

General clinical observations were made every day with specific rectal temperature/injection site observations and clinical observations made at day -1, day 0, 3, 5, 7, 14 and 28. Blood for serum was taken at Day-14 to confirm animals were still RVFV seronegative, Day -1 as the baseline and at Day 28 for the primary endpoint.

Table 5.1 Sampling types and collection timeline

	Study Day (n=180 per species)									Month (n=100 per species)			
Sample type	-14	-1	0*	1	3	5	7	14	28	3	6	9	12
Serum	✓	✓							✓	✓	✓	✓	✓
EDTA		✓		✓	✓	✓	✓	✓	✓				
Nasal		✓		✓	✓	✓	✓	✓	✓				
Rectal		✓		✓	✓	✓	✓	✓	✓				
PBMCs		✓						✓					
Clinical Observations	✓	✓	✓	✓	✓	✓	✓	✓	✓				
Injection Site Observations	✓	✓	✓	✓	✓	✓	✓	✓	✓				
Rectal Temperature	✓	✓	✓	✓	✓	✓	✓	✓	✓				

*(Day 0 was day of vaccination)

5.2.2. Sample size calculations

Sample size calculations were based on the primary endpoint analysis: a non-inferiority comparison of two binominal proportions to the seroconversion of RVF Gn antibodies in animals vaccinated with ChAdOx1 RVF and Smithburn. To calculate the sample size an assumption of 97% prevalence of seroconversion in all four species with 90% power and a significance level of 5% (two-sided test) and a non-inferiority margin of 10%. The non-inferiority margin was pragmatically calculated based on the potential consequence of low vaccination coverage on livestock and human health during RVFV outbreaks. Robust estimates of seroconversion to Smithburn vaccine in field conditions were unavailable but studies on a different licensed live vaccine with comparable immunogenicity to the Smithburn vaccine, Clone 13 indicated a seroprevalence of 97% in goats, 91% in sheep and 67% in cattle (Njenga et al. 2015). The sample size required was 62 animals per group. Due to the worries of loss to follow up through issues such as natural exposure to other diseases or predators the sample size per group was increased to 80 animals for the non-inferiority comparison.

5.2.3. Randomisation and blinding

Animals were randomly allocated to treatment groups but were balanced by sex, age and body weight as much as possible. The experimental unit was the individual animal. All field personnel involved with clinical examinations, injection site observations, general health observations, measuring rectal temperature, collecting and processing blood samples, nasal swabs, rectal swabs and data collection and allocating animals to treatment groups (ID tags) were blinded (this included myself). A statistician and a veterinarian were unblinded for allocating and administering the vaccines. Neither of these individuals were involved with examinations or collection of samples. Although this trial has a follow up study up to 12 months post vaccination, study animals were re-coded post Day 28 on the datasets so I could

analyse the data for this thesis and the study team could remain blinded for the 12 month follow up (i.e. animal ID tags were removed from datasets, a recoded unique identifier was given along with the treatment groups).

5.2.4. Recruitment

Sheep, goats and cattle were recruited from the Kapiti ranch, whilst the camels were recruited from several ranches in Laikipia (see Chapter 3). For animals to be included in the study they had to meet the eligibility and exclusion criteria (Table 5.2 and 5.3).

Animals on the Kapiti ranch are routinely vaccinated against a number of diseases (Table 5.4). Routine vaccination scheduling was conducted to meet the eligibility criteria for the field trial (Table 5.2). After discussions with the field trial team it was decided that, although the Malignant Catarrhal fever vaccine is not commercialised, it would still be part of the vaccination schedule for field trial animals due to the high risk of MCF transmission.

Table 5.2 Inclusion Criteria for non-inferiority field trial

Inclusion Criteria	
•	Healthy as determined by veterinarians at the study site prior to administering the vaccines
•	Amenable to handling
•	Weaned for a minimum of 4 weeks before enrolment into the study
•	At least 4 months of age and not knowingly older than 4 years
•	RVFV seronegative based on the ID Screen® Rift Valley Fever Competition Multi-species testing kit
•	Routine vaccinations should have been administered at least 4 weeks prior to Day 0 of the study (vaccination day) and subsequent boosters and medication should be administered from Day 60 (2 months post vaccine administration)
•	At the study site for at least one week prior to selection and to start acclimatisation at day -14

Table 5.3 Exclusion Criteria for non-inferiority field trial

Exclusion Criteria	
•	Unhealthy, injured and/or under-weight animals are explicitly excluded from the study unless agreed by the Monitor
•	All animals with hernias or other gross abnormalities will be excluded
•	Animals that have previously received of an investigational vaccine or product likely to impact on interpretation of trial data
•	Females that are either known to be pregnant or are lactating at the time of screening

Table 5.4 Routine vaccinations administered at the Kapiti ranch

Name	Disease	Company	Commercial (Y/N)	Use	Species
Fotivax	Foot and Mouth Disease	KEVEVAPI	Yes	Every 4 months	Cattle, sheep, goats
O-Manisa	Foot and Mouth Disease	Boehringer Ingelheim	Yes	Planned to be used	Cattle, sheep, goats
Lumpivax	Lump Skin Disease	KEVEVAPI	Yes	In case of disease threat in the neighbourhood	Cattle
Blanthax	Black Quarter and Anthrax	Coopers	Yes	Annually	Cattle, sheep, goats
Jovaclost T	Enterotoxaemia	Jovac	Yes	Annually	Sheep
MCF vaccine	Malignant Catarrhal fever	MoreDun Institute	No	Annually	Cattle

5.2.5. Vaccine administration

The ChAdOx1 RVF vaccine and PBS vials were produced at The Jenner Institute, University of Oxford under the principles of Good Clinical Laboratory Practice (GCLP). A liquid formulation of the ChAdOx1 RVF vaccine was used, though parallel studies have now demonstrated that the vaccine is also stable when lyophilised.

Permits authorising importation of the ChAdOx1 RVF and placebo vaccine into the country were submitted and approved by the Kenya National Biosafety Authority, the Kenya Pharmacy & Poisons Board and the Directorate of Veterinary Services in February 2019. A UK supplier of customised services to medical and life science research in LMICs, Carramore International Ltd. was used to transport the vaccines under cold chain and coordinate all customs requirements. The Smithburn (Riftvax™) vaccine was acquired from the local manufacturer Kenya Veterinary Vaccines Production Institute (KEVEVAPI). The vaccines were kept at ILRI main campus as there were reservations about keeping all the vaccines at the farm which runs off solar power. ILRI main campus freezers run off a centralised system with back-up generators. Cold chain was maintained throughout transportation to vaccination of animals.

Animals in the PBS group were vaccinated with a 2ml intramuscular dose which came in 10ml cryovials (four doses). The ChAdOx1 RVF group were vaccinated with 2ml intramuscular dose. Dosing was based on the success of the previous vaccine efficacy studies (Warimwe et al. 2016, Stedman et al. 2019). The ChAdOx1 RVF vaccine came in a 10ml cryovials which would contain four doses per vial. Each vial contained 5.0×10^8 infectious units per ml of ChAdOx1 RVF vaccine. Sheep and goats in the Smithburn (Riftvax™) vaccine group were vaccinated with 1ml dose subcutaneously, while cattle were vaccinated

with 2ml as directed per the manufacturers instructions. There was no reference dose to give to camels so it was decided they would be vaccinated with 2ml, similar to the cattle.

5.2.6. Sampling

Veterinarians sampled blood (serum, EDTA and PBMCs) from the jugular vein, as well as taking the rectal and nasal swabs (Figure 5.2). Nasal and rectal swabs were collected by gently rotating the swab around the mucosal wall of the nasal/rectal cavity. EDTA vacutainer tubes (Becton Dickinson, UK) were used to collect uncoagulated blood (10ml, purple top), plain vacutainer tubes were used to collect coagulated blood for serum (10ml, red top). Nasal and rectal swabs were taken using Flock swabs which were then given to myself and the veterinary intern to process in 2ml cryovial tubes of PBS. We also completed the data capture forms for the clinical observations, injection site, rectal temperatures and sample collection and identification.

Field sampling took approximately 2-4 hours to sample 90 animals, depending on the number of samples required (PBMCs in sheep and goats took substantially longer as two syringes were needed). After collection, samples were processed in the field lab following the schedule in Figure 5.3. Serum was obtained by spinning coagulated blood using a centrifuge at 2000rpm for 20 minutes. Plasma was obtained by spinning uncoagulated blood at 1500rpm for 15 minutes. Whole blood was aliquoted into 2ml tubes, one with trizol in it and one whole blood alone. Nasal and rectal swabs processed in the field were aliquoted into a separate tube so there were two aliquots. Two aliquots of serum were also made for resilience purposes. All samples were kept at -20°C in the farm laboratory facility freezer. Once shipped to KEMRI-Wellcome Trust Research Programme in Kilifi, they were kept at -80°C before testing.



Figure 5.2 Sampling camels and field trial set-up

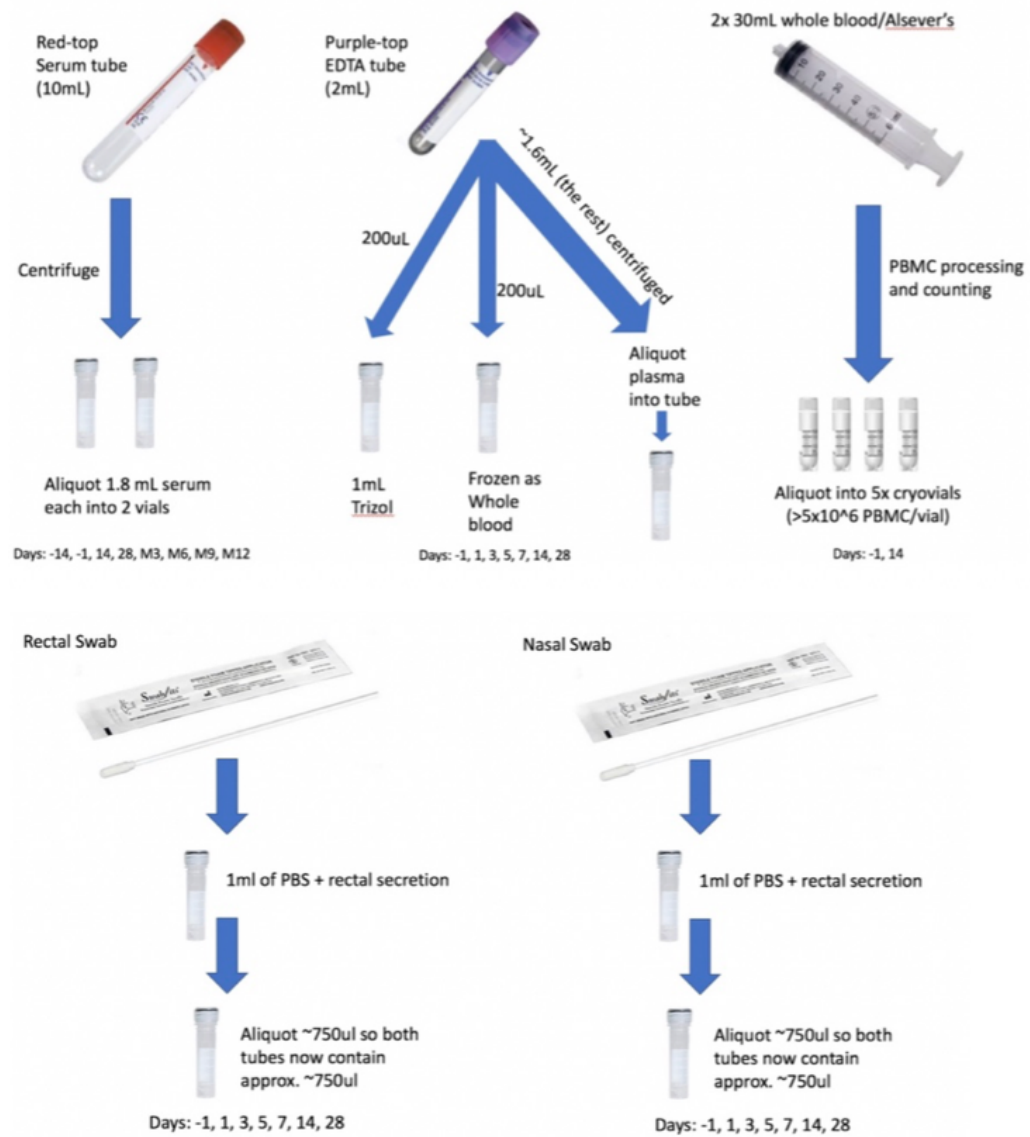


Figure 5.3 Sample processing flowchart

5.2.7. Data collection

Details of clinical observations data collection to assess vaccine safety can be found in Chapter 6. A list of all data collection forms completed for the trial can be found in table 5.5.

The Clinical and Injection site observation and rectal temperature form was used to record all observations that could potentially inform vaccine safety. At the start of the trial these were split as three separate forms. When sampling the sheep, it became evident the workload was too much having separate data collection forms for injection site reactions, clinical observations and rectal temperatures. These were then consolidated into one form for the remaining species and this was authorised as a protocol amendment. This improved operational efficiencies, as well as personnel being comfortable in their roles.

Table 5.5 Data Capture Forms

Animal Selection	recording animals that were sampled to potentially be recruited to the study
Animal Enrolment	animals which were recruited into the study and their demographic information
Daily Log of Events	used to record anything out of the ordinary associated with the trial (e.g. monitoring visits)
General Health Observations	record any health observations on days when clinical observations were not assessed
Accounting for vaccines (ChAdOx1 R/VF/Smithburn/PBS)	An important regulatory document, reporting on use of vials (used/part-used/unused)
Treatment Administration	Blinded document that records which animal received which vaccine (only colleagues who are unblinded have access to completed forms)
Clinical and Injection site observations and rectal temperature	Records all clinical data for assessing vaccine safety
Sample Collection and Identification	For regulatory requirements this document was important for registering what samples had been taken and their identification
Veterinary Report	If an animal was sick a veterinary report would be completed including details of any medication given
Temperature Records (Fridge/Freezer)	Temperature record forms were kept on fridge and freezer doors and completed twice a day (am/pm)
Mortality	Details and dates of animal deaths
Necropsy	Records of any unusual observations in different organs/tissues when conducting post-mortem
Animal Fate Post-Study	Record of what happens to animals when study finishes
Sample Reception Log	Outlines where samples can be found at KEMRI-Wellcome Trust Research Programme
Shipping Log	Form that is sent with samples when they are shipped outlining what is in each cryovial box

5.2.8. Data management

Following vGCP standards all data was collected on hard copies. Data was then uploaded onto an electronic database using the Epicollect5 software (Aanensen et al. 2009) by data clerks. I performed the data cleaning for day 28 analysis and then downloaded the datasets from the Epicollect software as Microsoft Excel files and sent them to the ILRI unblinded statisticians to recode and include treatment allocations for analysis. To meet requirements for registration the hard copies have been kept and archived. Information on data analysis for vaccine safety can be found in chapter 6 and for non-inferiority in chapter 7. The Consort statement was used as a standardised method of reporting how the non-inferiority study was conducted (Appendix A5.2) (Piaggio et al. 2012).

5.2.9. Ethics and Regulation

The importance of science communication, regulation and working with national government officials was paramount to implementing this study. In Kenya the regulation of veterinary vaccines is facilitated through the Directorate of Veterinary Services (DVS) which sits under the Ministry of Agriculture, Livestock, Fisheries and Cooperatives. In the case of viral vectored vaccines, the National Biosafety Authority (NBA) of Kenya also regulates vaccine development through its mission of ensuring safe development, transfer, handling and use of genetically modified organisms. As the ChAdOx1 RVF vaccine has been genetically-engineered it falls under the remit of the NBA and hence an application to conduct the trial was submitted through these officials.

In October 2016 the study team hosted the first meeting with these government officials at the ILRI Kapiti ranch. Here, the officials were shown the study site, the provisions the farm had and were able to discuss with them their concerns. Following on from this meeting the approval was given provided certain conditions were met:

1. Import permits for the vaccine are obtained from DVS who will ensure the vaccine is well packaged and securely transported from the airport to ILRI
2. The Confined Field Trial (CFT) site must be inspected by NBA and DVS before the commencement of the trial
3. Provide operational manuals/SOPs on biosafety and biosecurity measures before commencement of trials
4. A representative from NBA and DVS must be present during the vaccination and the disposal of the unused vaccine as well as the destruction of all livestock samples following the termination of the trial
5. A detailed schedule of activities and the experimental design must be provided both to NBA and DVS before commencement of the trial
6. Put and implement measures to ensure that no transgenic material from the laboratory and the CFT enters the human food or animal feed chain. Contingency measures should also be put in place in case of accidental escape of the trial animals under confinement
7. All the trial animals and materials must be rendered biologically inactive through autoclaving and or incineration. Strictly adhere to the proposed waste management plan and records must be maintained and availed to biosafety inspectors on request. Any material retained for further analysis shall be counted and accounted for at all times.
8. The applicant must work with the Department of Veterinary Services to produce the data required for vaccine registration
9. Provide quarterly and annual progress reports to NBA in the prescribed format. The reports should be discussed by and forwarded through the Institutional Biosafety Committee.

10. All staff involved in this trial must be trained by DVS and NBA officers on handling transgenic vaccines and overall biosafety and biosecurity matters before commencement of the trial.

The approval letter can be found in Appendix A5.3.

A rebuttal was sent to the NBA and DVS to question approval condition 4 and 7. It was important we communicate that these samples are useful for many research purposes and we should not be required to destroy them at the termination of the trial. Further to this the majority of these animals would be given a commercialised vaccine or a placebo and kept in a normal farm setting, and thus culling was not warranted specifically for these animals. We understood they would want to see vaccine shedding data on the animals vaccinated with the investigational ChAdOx1 RVF vaccine before deciding if incineration was necessary for these animals. In April 2017 the NBA confirmed they were happy with our suggestions and agreed to amend approval conditions 4 and 7 to facilitate these comments.

In March 2018 the NBA and DVS visited the farm to conduct an inspection of the site. Further recommendations were made including GPS tagging animals and putting 'restricted access' signage at the entrance of the confinement area. The officials were conscious of lack of fencing around the site. This led to a group discussion to provide rationale and actions where needed:

- The farm has a wildlife corridor running through it and this has to be kept open for conservation reasons.
- Fencing is a commodity and often gets destroyed and removed.
- The farm does have trenches around the perimeters.

- All study animals are kept in herds which are managed by animal herders throughout the day. In the evening they are kept in bomas (enclosures) which are patrolled by security guards at night.

A final pre-trial visit with the NBA and DVS officials was conducted on 20th March 2019, whereby they confirmed the trial could commence. They recommended access signage was more secure (Figure 5.4) and training certificates were produced for all the animal herders which was followed up soon after the meeting and before starting the trial.



Figure 5.4 Entrance to confined field trial area with access signage at Kapiti plains ranch

As ILRI was subcontracted to do the field study, ethical approval was sought from their animal ethical review board: The Institution Animal Care and Use Committee (approval number: 2018-07). As the Pirbright Institute (TPI) was the sponsor and the principal investigator was based at KEMRI-Wellcome Trust Research Programme where samples are kept, approvals were also needed from these institutes. During the process it was realised there was a lot of repetition from the different institutional ethical boards. After discussions we were able to set up reliance agreements between TPI and ILRI and KEMRI-Wellcome Trust Research Programme and ILRI.

The study followed the Extension of the Consort 2010 statement for reporting of non-inferiority and equivalence Randomised Trials (Piaggio et al. 2012) (Appendix A5.2) as well as the Animal Research: Reporting of In Vivo experiments (ARRIVE) guidelines (Kilkenny et al. 2010)

5.2.10. Study sites and personnel

The study was conducted across three locations. The field trial took place on the ILRI Kapiti Plains Ranch Ltd. A more detailed description of the farm can be found in section 3.2.1. As outlined by the NBA application and approval conditions, the field trial was conducted in a confined area of the farm. This was a separate area of 9,000 acres away from the working farm animals (Figure 5.5). Although the trials were staggered by species the follow up duration of antibodies study (not part of thesis) meant there would eventually be a large number of all four species in the area (~400 animals). Furthermore, animals were not allowed to be released from the area until approval had been granted by government officials, despite some animals being dropped from the study after the first 28 days. The large area assigned to the trial meant there was enough space for grazing, rotating bomas and in case of any issues such as flooding and drought.

The ILRI main campus laboratories were used for processing PBMCs. This was because biosafety cabinets are needed to process these samples and the farm does not have this equipment. Blood samples were couriered to ILRI main campus on the same day as sampling to be processed at their laboratories. The definitive assays were carried out at KEMRI-Wellcome Trust, Kilifi along with data entry and this is currently where all hardcopies of data collection forms are archived. Field trial samples were couriered directly from the Kapiti farm to KEMRI-Wellcome Trust which would roughly take 8-12 hours to drive (Figure 5.6). During busy sampling time periods (e.g. the first 7 days of vaccination) samples were collected regularly. Samples would not remain on the farm for more than one week as it was deemed a risk due to the potential issues that may occur using solar power for electricity. Both ILRI main campus and KEMRI-Wellcome Trust run off mains electricity with more sophisticated infrastructure (e.g. Comark temperature monitored freezers).

Table 5.6 outlines field trial roles and how they were split across sites. Following vGCP and monitoring public health clinical trials a duty delegation list was implemented which recorded all study roles and responsibilities. In addition to the core field trial roles three extra herders were hired on a casual basis due to their expertise in handling camels during the camel sampling period.



Figure 5.5 Map of ILRI Kapiti Plains ranch. Confined field site is shown in the red circle.

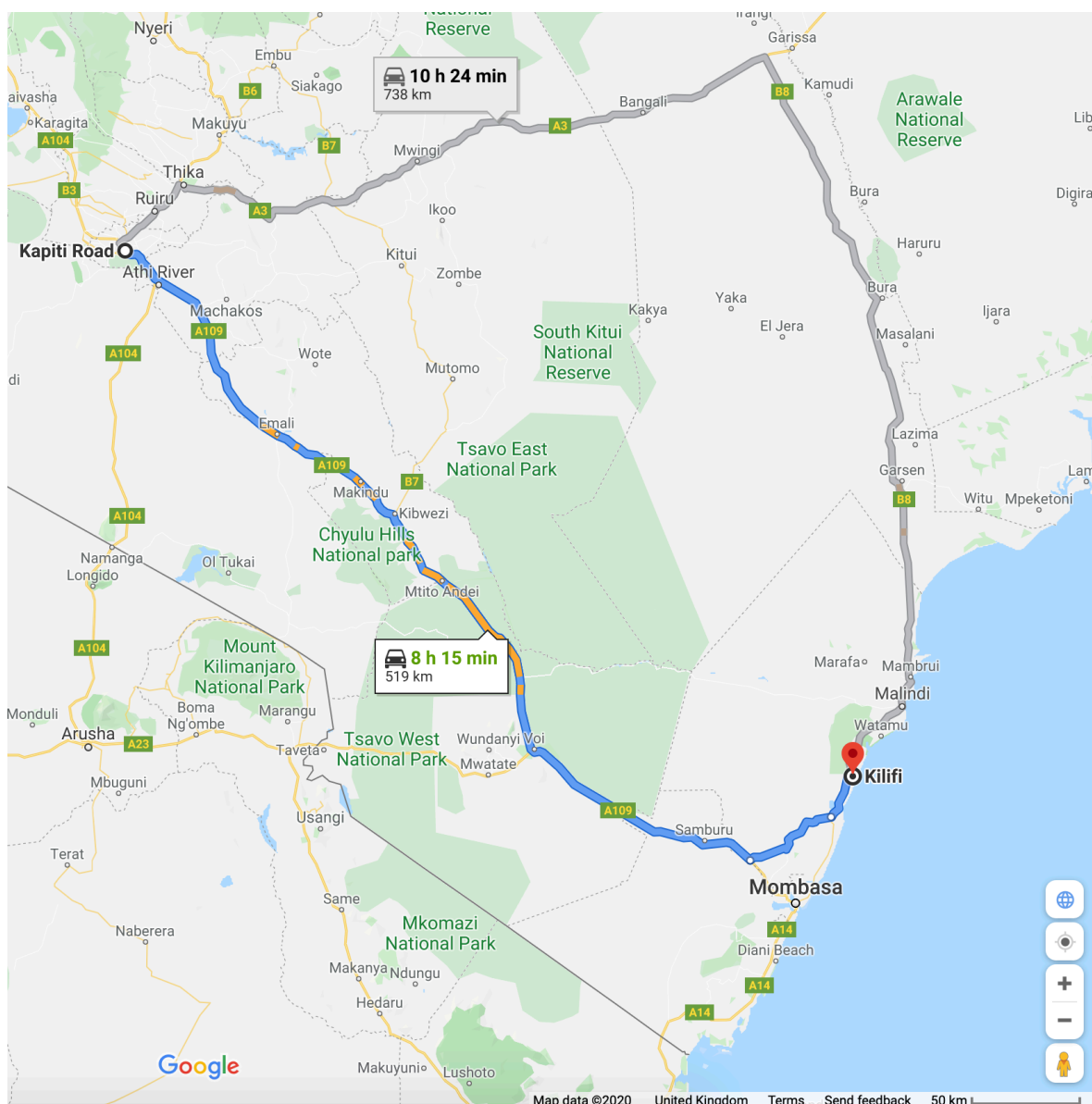


Figure 5.6 Distance between Kapiti ranch (field trial site) and KEMRI-Wellcome Trust in Kilifi. Google maps accessed 21/04/2020.

Table 5.6 Field Trial Roles

MANAGEMENT TEAM	SPONSOR	INVESTIGATOR	MONITOR
COORDINATION ACROSS SITES	Trial Coordinator (This was my role)		
SITE SPECIFIC	ILRI (Field Team)	KEMRI Wellcome Trust	
	Veterinarians (x3)	Clinical Trials Monitoring Team (x2)	
	Veterinarian intern (x1)	Data Manager	
	Field lab technicians (x2)	Data Clerks (x2)	
	Rotated Animal Technicians/herders (x6)	Lab Technicians (x4) - Sample Manager - PCR - Immunology - Cellular	
	PBMC lab technicians (x5) (ILRI main campus)	Trial administrators (x2)	
	Unblinded Statisticians (x2)		

5.2.11. Monitoring

The clinical trials monitoring team used a defined protocol to examine activities conducted for public health clinical trials. I worked closely with the team to draft the monitoring plan and checklists for the field trial making sure we met vGCP standards. The plan included site initiation visits to check the site was fit for purpose, routine visits during the trial, monitoring all field trial documentation and data collection forms. I was able to identify important differences between veterinary and human medicine.

In public health clinical trials, a Trial Master File (TMF) is a collection of all the essential documents associated with the trial. When implementing the field trial, a TMF was developed based on checklists monitoring teams used for human trials. Certain documents were adapted to suit veterinary practice. In human medicine anyone participating in clinical trials should give informed consent to take part. With livestock animals this cannot be achieved, but the protocol was submitted to the ILRI and Pirbright animal ethical review boards. Where we leased camels from other farms, a leasing agreement stipulating how the animals would be cared for and information about the trial was signed by the camel owners and the ILRI legal team, confirming permission of use and stewardship, respectively. The government official training and the ethical approvals further support animal welfare requirements. Appendix A5.4 outlines what was included in the Trial Master File. In human clinical medicine an investigator's brochure is a compilation document which summarises all clinical and nonclinical data on the investigational product. This is a document that provides supporting information to the protocol and is not routinely produced for veterinary trials. An investigator brochure was created by colleague Danny Wright, The Jenner Institute, University of Oxford for the field trial.

5.2.12. Training

All personnel involved with the trial had training on the protocol. All field trial personnel attended a three-day training course on animal welfare in research and handling GMOs by the NBA and DVS (Approval condition). I, as study coordinator, provided protocol training with personnel who were proficient in English. The Kapiti veterinarian repeated this in Swahili for animal technicians and herders for reinforcement. All Kapiti animal technicians also had training on camel handling specifically as camels were being brought to the farm for this project.

5.3. Successes and challenges implementing a vaccine field trial

The field trial commenced in March 2019 and the sampling for this thesis was completed in October 2019 (Table 5.7 Sampling Timeline). During the field trial 180 sheep, goats and cattle were successfully vaccinated and sampled up to Day 28 post-vaccination. Due to sourcing seronegative camels and the logistics required in sampling, a two-phase approach was implemented for this species. In September 2019, 73 camels were enrolled into the study, vaccinated and followed up (ChAdOx1 RVF group n=32, Smithburn group n=32, Placebo group n=9). The second round is due to begin in the first quarter of 2020 (not included in the thesis).

One animal per herd wore a GPS collar (Figure 5.7) in line with NBA approval conditions, to make sure they stayed in the boundaries of the confined field area as well as for insurance purposes. All eligible animals were successfully recruited to the study (see Chapter 3). The study was staggered by species starting with sheep in April, goats in May, cattle in July and camels in September 2019. When drafting the gantt chart we had scheduled cattle to be vaccinated and sampled in May. We were grateful one of the trial veterinarians, Dr Annie Cook, is a Malignant Catarrhal Fever (MCF) expert had raised concerns about conducting

the cattle trial at that time. Wildebeest calving season is in May and at birth wildebeest calves are viraemic for MCF and thus when in close contact the virus can be transmitted to cattle. We therefore swapped the schedule so that goats were sampled in May. This came with some additional issues. The goats were very young and small in size, although still meeting the eligibility criteria (older than 4 months of age). There were worries that taking two 10ml syringes for PBMCs would be over the blood volume limit and could have animal welfare consequences. To mitigate this, I calculated the blood volume limit based on size. Where goats weighed enough, we took two syringes, where they did not, one syringe was taken. All goats weighed enough to have at least one syringe.

Table 5.7 Sampling timeline up to the primary endpoint analysis (28 days post-vaccination)

2019							
Species	Mar	Apr	May	Jun	Jul	Aug	Sept
Sheep (n=180)							
Goats (n=180)							
Cattle (n=180)							
Camels (n=73)							



Figure 5.7 Sheep with GPS collar on and satellite map of GPS tracker for 3 GPS tagged animals.

Each individual sampling of an animal would take on average 3 minutes (5 minutes if PBMCs were taken). This meant sampling would approximately take 4-5 hours. After this, samples needed to be processed which would take another 2 hours approximately.

A large challenge during the set-up of the trial was procuring all the consumables and reagents needed for the study. Both ILRI and KEMRI-Wellcome Trust have a contract with Carramore whose expertise lie in sourcing and shipping life science consumables and equipment to resource limited settings. However, issues with customs permits meant there was over a 6-month lead time for some items. Having several institutes involved with the project meant we were able to source consumables last minute from ILRI or other labs. The number of samples this field trial generated meant there were also freezer space issues at KEMRI.

During the field trial we faced one overnight failure with the generator that caused the field laboratory fridge and freezer to start defrosting. For this situation I arranged for the courier to come the same day to take the samples straight to KEMRI-Wellcome Trust.

The operational efficiency of the field trial teams improved over time, due to experience, confidence and adapting procedures to make them more suitable. A pre-trial site visit was conducted with the monitoring team. The monitoring team reported that it was very helpful to witness how a typical sampling day worked in terms of time with individual animals, how safety reporting was carried out, timings and processing samples. To understand the data in full all personnel should visit the study site and observe a sampling day. Unfortunately, the data manager and data clerks did not get to observe a sampling day but I feel this would have benefited them when uploading and cleaning data.

Although all field trial personnel were aware of sampling collection timings and shipment, the vast number of samples collected produced a huge strain on freezer space at KEMRI-Wellcome Trust. This amplifies the need for strict planning measures at a laboratory/institute level.

During the study a number of protocol amendments and deviations were recorded. Several deviations occurred with the day -14 sampling. For camels, this was due to staff shortages and for cattle it was due to human error by sampling a day earlier. In the case of the deviation for sampling the cattle at Day -15 being a human error, it was decided that these samples would be used as it would not have any significant impact on the study and was suitable for animal welfare reasons (by not repeating blood sampling unnecessarily. Two amendments were made, the first was to combine the clinical observations, injection site reaction and rectal temperature records on to one form to save on time and errors. The second was the eligible age of animals used, confirming goats aged 4 months and above were eligible to be used in the study. A small number of human errors occurred during the course of the trial, emphasising the importance of protocol training with field trial staff as well as with general farm personnel. The most common error was forgetting to strike, initial and date any mistakes made on forms in line with vGCP. Other errors included making sure thermometers were disinfected between animals or forgetting to record rectal temperature/clinical/injection site observations although this occurred very few times.

5.4. Conclusions and lessons learnt

This study was an exemplar trial conducted on a One Health platform. Although a veterinary trial, a human clinical trials monitoring team monitored the trial and all samples were stored and analysed in a laboratory that primarily works on human clinical trial samples. Much was learnt from conducting the trial this way. Although difficult to directly compare, this vaccine

field trial was run at a comparable level to phase II clinical trials in a public health setting. This included assessing both safety and effectiveness, the number of animals involved and the level of monitoring activities.

In Kenya viral vectored vaccines are classed as Genetically Modified (GM) as the viral vector has been genetically engineered. Although the NBA have regulated a number of small-scale veterinary vaccine trials in high containment facilities, this trial was the first GM veterinary vaccine field trial to be regulated through the NBA in Kenya. Through the implementation process it was evident that both the DVS and NBA support the vaccine technology and want the trial team to collaborate with them through the vaccine registration process (an approval condition). The NBA's main experience has mostly been on plant GMOs meaning NBA checklists were more directed at crop research rather than vaccines. Pre-trial meetings were a useful tool to communicate the trial aims and provide understanding to the regulators. As part of the approval condition NBA had to accompany the vaccines whenever they were moved (airport to ILRI main campus, ILRI main campus to the farm) as well as be present during vaccination. However, there was little formal monitoring and regulation on the ground, suggesting the need for more capacity on monitoring and regulation in these government departments.

This trial has also demonstrated the need to have improved systems and capacity for procuring laboratory consumables into countries, particularly in Africa. It is widely acknowledged anecdotally in many laboratories across Kenya that timings between ordering and receiving consumables can take multiple months, when in the UK the same consumable can be delivered overnight. Similarly, stable infrastructure, such as electricity, is needed in field laboratories for trials that are working to vGCP, being monitored and which research may be used in vaccine registration dossiers.

Reliance agreements are highly suitable when working across multiple sites on large scale field trials. The reliance agreements put in place between the multiple institutes meant there was no repetition in submitting applications to ethical boards or reporting. It also meant the field trial protocol was approved from two veterinary health institutes, ILRI in Kenya and The Pirbright Institute in the UK and by a public health institute, KEMRI-Wellcome Trust. The trial therefore met Kenyan national standards as well as UK ones with regard to animal ethics and welfare. Such agreements and partnerships have been seen for other novel field trials including Ebola vaccine trials (Mooney et al. 2018). The reliance agreement provided ownership to the host institute of the trial, but also provided binding agreements from other affiliated parties. Similar to public health trials, although protocols are to be followed, there does need to be a level of flexibility when issues arise. One of the biggest aides in running large trials is the constant dialogue and communication between partners of the project and owning roles. This is a large factor for the successful running of the trial (Mooney et al. 2018).

The veterinary vaccine regulation process does not use the extensive clinical trials process. In human vaccine development quality, safety and efficacy is evaluated before entering into any human clinical trials, which may include using animals as models. Human clinical trials evaluate safety and efficacy of the investigational product with a gradual increase in participants and field studies are conducted to assess effectiveness. In veterinary vaccine regulation authorisation is often based on laboratory experiments alone including those in high containment. Where possible, field trials are conducted to provide supporting data however these are not as standardised or extensive as human trials. Here, monitoring processes have been used to standardise the trial. A much larger sample size was used compared to laboratory experiments, the results provide a more realistic depiction of vaccine

safety, immunogenicity and effectiveness in target animals, and under natural field conditions.

The implementation of the field trial was a learning curve in a number of ways. The scale, personnel and systems in place needed to run such a trial was not fully appreciated in terms of sampling, completion of documentations and quality assurance. In order to run successful vaccine field trials that provide suitable data for registration and licensure dossiers vGCP principles need to be in place. This trial has shown what a significant undertaking it is to make sure the trial and results are produced to this standard. The vGCP document is very important to follow but the practicalities of implementing such recommendations lacks in guidance. If research organisations are going to have more involvement in the veterinary clinical trial process a proposal would be to form working groups involving researchers, regulators, industry and funders to help plan these trials practically. Forums such as the OIE or the International Veterinary Vaccinology Network (www.intvetvaccnet.co.uk) would be suitable organisations to support this.

In recent years the threat of emerging disease outbreaks has become more of a worry to the general public. Many public health emergencies such as Ebola, Rift Valley fever, Middle East Respiratory syndrome and Nipah are zoonotic diseases. The most recent of these is the Coronavirus, COVID-19, from live animal markets in Wuhan, China, where transmission has occurred at an alarming rate internationally (Heymann and Shindo 2020). In addition, the recent African Swine Fever epidemic affecting pigs and wild boars has had a huge effect on the economy and peoples' livelihoods in Asia (Van Der Zee, 2019).

Unlike human vaccine development, which is overseen by public bodies, the private sector is the main source for funding veterinary vaccine trials and there is therefore limited funding

available and coordination to run large vGCP vaccine field trials (Knight-Jones et al. 2014). These outbreaks may aid in awareness and support for a paradigm shift with veterinary vaccine regulation. Paradigm shifts in the vaccine regulation process in vaccines for public health use have already been implemented to fast track vaccine testing during the devastating Ebola outbreaks in Western Africa in 2013 (Gsell et al. 2017). Specialised coalitions such as CEPI (a global partnership between public, private, philanthropic and civil society organisations) have been set up in the wake of these outbreaks to accelerate development of vaccines for emerging infectious diseases and enable equitable access to these vaccines for people most in need. Although the emphasis is on delivering public health needs, a number of veterinary vaccine field trials have been funded through these channels: to develop vaccines for target animals that acquire the disease first and so halt part of the transmission pathway; for providing robust data in ‘real-life’ field context; and as pre-clinical trials that can easily be translated into human medicine which is specifically important for a One Health disease such as RVF (Füördö et al. 2015).

In the veterinary context GALVmed specialises in product development partnership: their vision is to improve the well-being and economic progression of smallholder farmers through livestock disease control. As well as funding veterinary vaccine development including vaccine field trials, they have also spearheaded a harmonised regulatory process for veterinary vaccines across Kenya, Tanzania and Uganda. One of the outputs from this is a mutual recognition procedure for obtaining marketing authorisation making it easier to register and market veterinary vaccines across east African countries (GALVmed 2016).

6. A non-inferiority Rift Valley fever vaccine field trial to assess vaccine immunogenicity in multiple livestock species in Kenya: Vaccine safety

6.1. Background

Safety reporting is a requirement in the registration and licensure process for veterinary vaccines (live and inactivated) and all veterinary vaccines must assess safety and regulatory requirements before applying for market authorisation as a commercial product. International guidelines have been produced by the European Medicine Agency (European Medicines Agency 2008) to provide criteria and recommendations for evaluating safety of live and inactivated veterinary vaccines in target animals. In order to meet registration requirements general clinical observations, rectal temperature, other performance measurements of interest and injection site reactions should be examined and reported at appropriate frequencies post-vaccination. All adverse reactions should be reported in the report which is produced for the regulators. The EMA have also produced guidelines on veterinary Good Clinical Practice (vGCP) which emphasises good clinical practice for veterinary trials, providing international ethical, scientific and practical assurances of how the studies are conducted and data produced.

As established in chapter 4 the safety profile of the ChAdOx1 RVF vaccine to date in laboratory safety studies is encouraging (Warimwe et al. 2016, Stedman et al. 2019). The first stage in evaluating vaccine safety before initiating field studies is laboratory safety studies (European Medicines Agency 2008). Both studies assessed safety by measuring rectal temperatures and general health observations over the course of the experiments and neither reported adverse events from the vaccine.

As recommended by the EMA regulatory guidelines both of the previous laboratory ChAdOx1 RVF trials have been conducted with small groups of animals ($n \leq 8$) (Warimwe et al. 2016, Stedman et al. 2019). In this chapter vaccine safety is assessed for the vaccine groups that are part of the non-inferiority field trial (Chapter 5 and 7). Hence, a much larger number of target animals were tested in a natural field setting. Adverse events were assessed for injection site reactions,

clinical observations and rectal temperature, as well as any general health observations over the first 28 days post-vaccination (Table 5.6 outlines what was reported).

A limited number of livestock vaccine studies conducted in disease endemic settings have been identified in the literature reporting use of veterinary Good Clinical Practice (vGCP) as part of the study design or monitoring by external monitoring teams (Njenga et al. 2015, Lyons et al. 2019). A randomised controlled field trial testing the safety and immunogenicity of another RVF vaccine, Clone 13 did emphasise that the study was conducted in accordance with European Medicines Agency recommendations for clinical trials and had an independent monitoring team assess the trial (Njenga et al. 2015). However, the paper did not provide a comprehensive account of the safety of the vaccine in the trial animals: rectal temperature was only taken on day 0, 1, 2 and 3 post injection; only when an animal developed a clinical condition did veterinarians provide clinical examinations and no details of this were reported; furthermore, there was no mention of injection site reaction being observed and reported.

This trial aims to comprehensively assess the safety of both the novel ChAdOx1 RVF vaccine and the commercialised Smithburn vaccine in multiple livestock species to the principles of vGCP. By conducting the trial to vGCP the immunogenicity and safety results would be of high ethical and scientific quality providing assurance and integrity of this data for vaccine registration and licensure purposes (European Medicines Agency, 2008).

6.2. Methods

For details on the study design please refer to Chapter 5.

6.2.1. Animal Welfare

Withdrawal criteria for animals enrolled in the study included:

- Any abnormal signs compromising the welfare of the animal
- Rectal temperature over 40.5°C for the fourth consecutive day
- Anorexia on the 3rd consecutive day
- Blood in the faeces on the 3rd consecutive day
- Animals that required concurrent medication that may compromise its suitability for the study

The field trial veterinarians were responsible for any animal welfare issues including removal from the study. The principal investigator, monitor and sponsor were informed as soon as decisions were made, and this was documented on specific document collection forms. Post-mortems were conducted for all animals that died.

6.2.2. Data collection

Safety data were recorded at day -1, day 0, 1, 3, 5, 7, 14 and 28. At vaccination (day 0), rectal temperature, clinical and injection site reaction observations were recorded twice: prior to vaccination and at least four hours afterwards. The veterinarians observed any clinical or injection site observations and also took the rectal temperature.

All core field trial personnel (veterinarians, coordinator, veterinary intern, animal handlers and field lab technicians) were blinded apart from the one veterinarian who administered the vaccinations who would not record any of the clinical observations to avoid bias.

Clinical observations were recorded using a scoring chart which assessed: depression, appetite, lameness, nasal discharge, respiratory distress, coughing and body condition. Injection site observations were also recorded. The veterinarians would report if there was an injection site reaction and then if the site had any heat, pain, reddening or hardening as well as the length, width and depth of the reaction (Table 6.1). This was drafted by the monitor Dr Michael Francis due to his expertise of working on R&D in industry. The clinical observation scoring guide which provides the rationale and case definitions for each score can be found in appendix A5.5. Rectal temperatures were also taken to measure any fever that may be associated with vaccine administration.

Table 6.1 Clinical observations, injection site reactions and rectal temperature reporting form

Date: (DDMMYY)		Start Time: HH:MM 24hr Clock)		End Time: (HH:MM 24hr Clock)		Species:		Sex: ☐ M ☐ F		Study Day: If Day 0: Pre-vaccine ☐ Post-vaccine ☐								
		Injection Site					Clinical Observations											
						Measurements (mm)												
Animal (ID)	Injection Site Reaction?	Pain?	Reddening?	Hardening?	Heat?	Length (XX)	Depth (XX)	Width (XX)	General Condition	Depression	Respiratory Distress	Cough	Nasal Discharge	Appetite	Lameness	Rectal Temperature	Time	Comments
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													
	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N													

6.2.3. Data analysis

Recoded data were initially retrieved from ILRI statisticians on Microsoft Excel. All statistical analysis was performed using the R statistical software environment (R Core Team. 2013).

Safety data including records of injection site reactions, clinical observations and rectal temperatures were initially assessed by number of animals in each treatment to have reported adverse events at least once during the study period. All variables in table 6.1 were assessed as adverse events that may be associated with vaccination. Any variables recorded multiple times in the comments column of the tables were also assessed for any significant association with vaccination group.

Fisher's exact tests were used to assess whether the frequency of adverse events within the first 28 days post-vaccination differed amongst treatment groups. For nasal discharge and lameness, where differences were reported, binomial-family generalised linear mixed models were used to assess differences accounting for factors other than treatment group. A generalised linear mixed model was chosen as it accounts for fixed effects such as treatment group, study day, age, sex, weight as well as random effects such as animal ID. The mixed models were performed using the lme4 package in R (Bates et al. 2015).

6.3. Results

6.3.1. Injection site reactions

Animals with an injection site reaction would always have swelling in the area. If present, other characteristics such as pain, reddening, hardening and heat would be recorded. There was no significant difference between vaccination arms in the frequency of animals with injection site reactions in any of the four species (Table 6.2). However, the frequency of injection site reactions did differ amongst species with sheep and goats having the most frequent reactions. Injection site reactions were only reported in one bovine (hardened injection site) and one camel (no other characteristics than a reaction), both of which were a single event.

Injection site reactions were reported in five goats (Figure 6.1): four goats vaccinated with the Smithburn vaccine, two of which had hardened injection site reactions and two goats vaccinated with the ChAdOx1 RVF vaccine (R1709, R2225), one of which had a hardened injection site (R2225) whilst the other had no other characteristics than a reaction (R1709). One goat vaccinated with the ChAdOx1 RVF vaccine had swelling at the injection site on Day 0 (R2225) and the other goat had swelling on Day 3 (R1709) only. The two Smithburn vaccinated goats (Animal ID R4279, R3369) were reported as having swelling at the injection site for multiple time points. One goat (R4279) experienced swelling at every sampling timepoint for the first 5 days (reported at Day 0 post-vaccination, Day 1, Day 3 and Day 5); on day 0 post-vaccination and day 1 the injection site was hard. Reported swelling of injection site for the other Smithburn vaccinated goat was on Day 3 and Day 5 (R3369).

Table 6.2 Number of animals (by species) with injection site reactions (%)

Species	ChAdOx1 RVF	Smithburn	Placebo	p-value†
Sheep	17 (21.3)	11 (13.8)	4 (20)	0.2
Goats	2 (2.5)	4 (5)	0 (0)	0.3
Cattle	0 (0)	1 (1.3)	0 (0)	1
Camels	1 (3.1)	0 (0)	0 (0)	1

† Comparison of treatment groups for each species

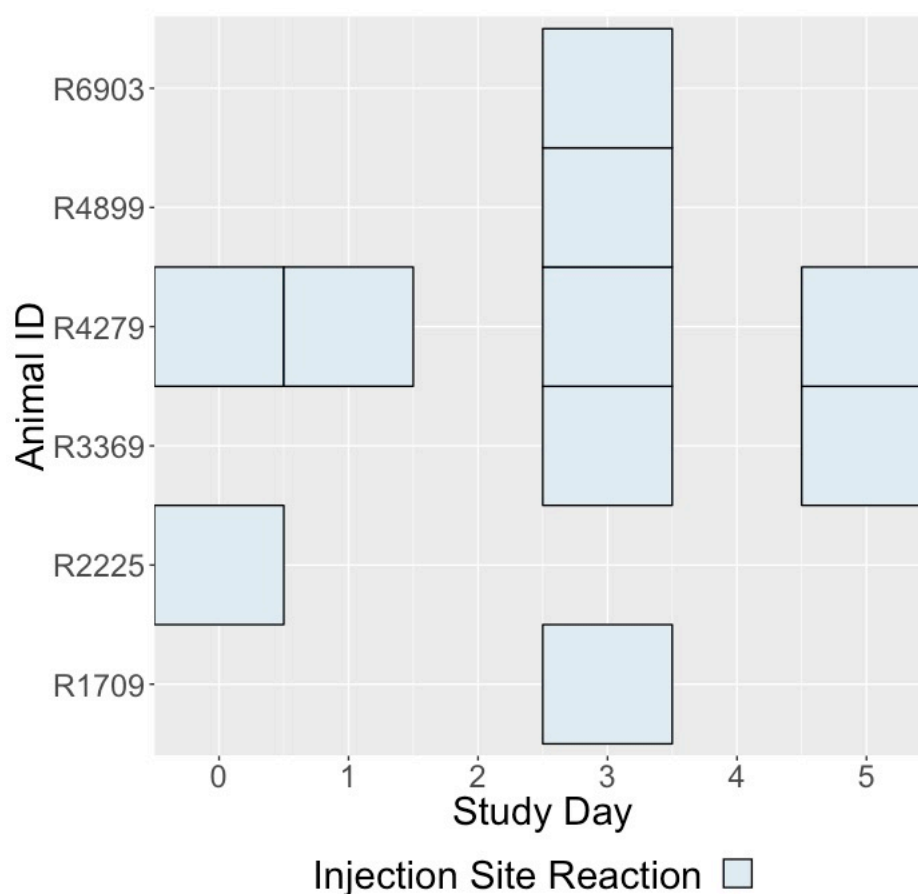


Figure 6.1 Goats showing injection site reactions (y-axis) and days on which they occurred (x-axis). Goat R1709 and R2225 were vaccinated with ChAdOx1 RVF vaccine, while goats R4279, R3369, R4899, R6903 were vaccinated with Smithburn vaccine.

Injection site reactions were most frequent in sheep, with multiple events observed in animals vaccinated with ChAdOx1 RVF (Figure 6.2) and Smithburn vaccines (Figure 6.3). Injection site reactions in sheep in the placebo group were less common (one on day 0; three on day 7). Overall there were 63/1259 events (5%) which reported injection site reactions irrespective of treatment group. Table 6.3 reports the number of sheep by treatment group to report injection site reactions and any pain, hardening, heat or reddening. The majority of injection site reactions had a hardening of the site (76.5% reactions for ChAdOx1 RVF animals, 81.8% of reactions for Smithburn vaccinated animals and 75% reactions for the placebo group).

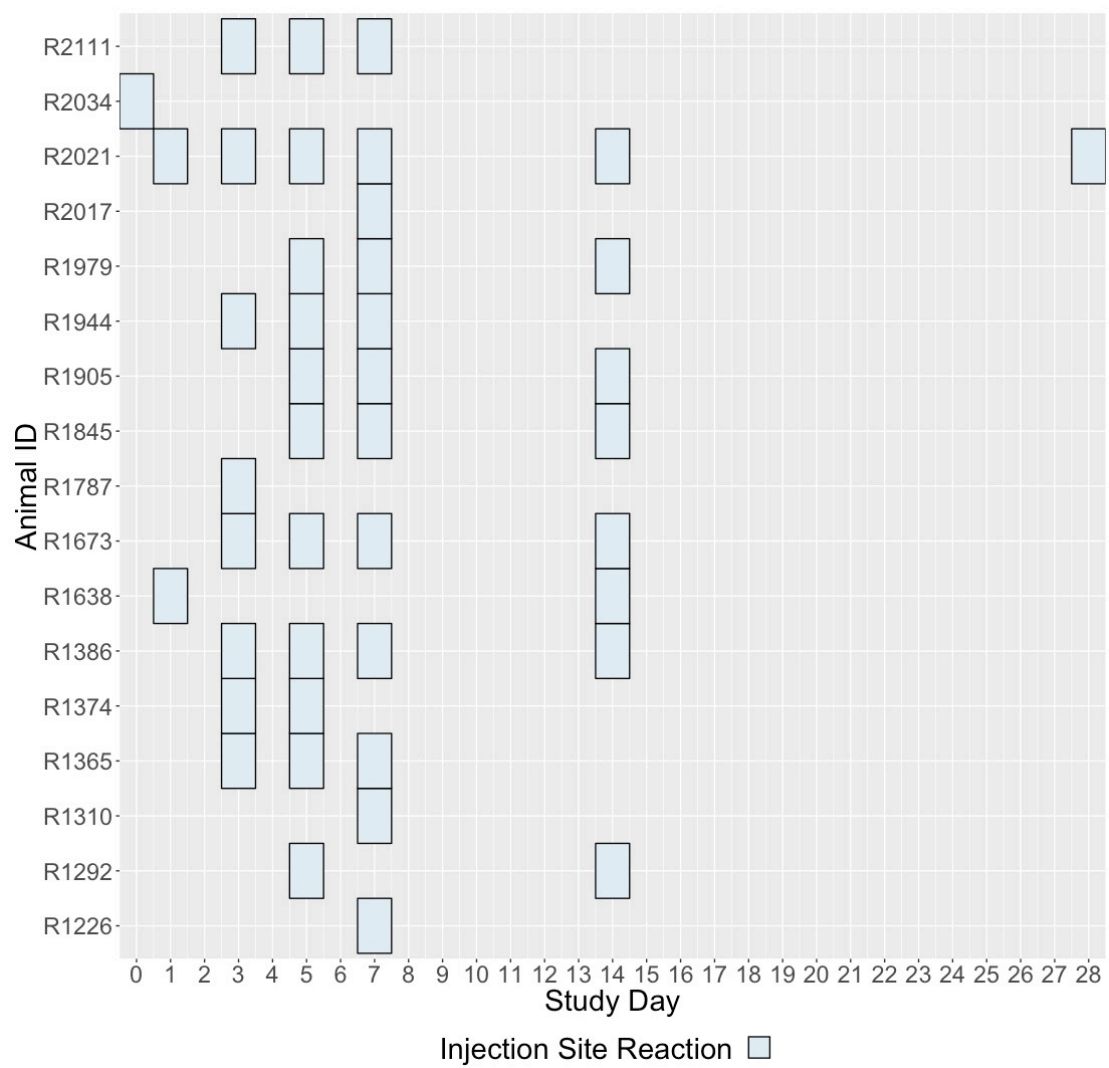


Figure 6.2 Sheep vaccinated with ChAdOx1 RVF showing injection site reactions (y-axis) and the days on which they occurred (x-axis).

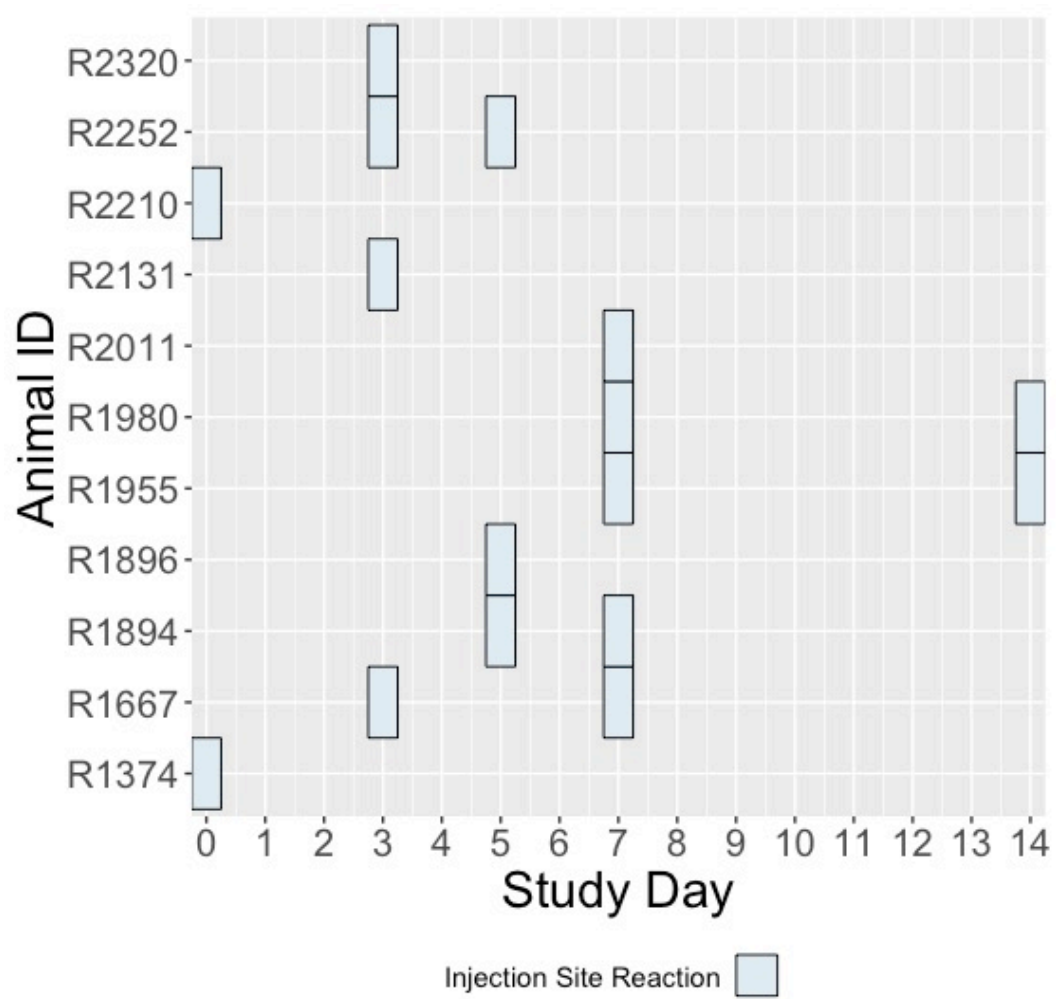


Figure 6.3 Sheep vaccinated with Smithburn showing injection site reactions (y-axis) and the days on which they occurred (x-axis).

Table 6.3 Number of sheep reported to have each type of injection site reactions (%)

	ChAdOx1 RVF (n=80)	Smithburn (n=80)	Placebo (n=20)
Injection site reaction (Swelling)	17 (21.3)	11 (13.3)	4 (20)
Pain	1 (1.3)	0 (0)	0 (0)
Reddening	1 (1.3)	2 (2.5)	0 (0)
Hardening	13 (16.3)	10 (12.5)	3 (15)
Heat	2 (2.5)	3 (3.8)	0 (0)

6.2.2. Safety results: Rectal Temperatures

Rectal temperatures were taken at the same time intervals as the clinical and injection site observations over the first 28 days post-vaccination (Day -1, 0, 1, 3, 5, 7, 14, 28). The median rectal temperature and interquartile range (IQR) were calculated for each treatment group on every day of sampling (Figure 6.4-6.7). No animals needed to be withdrawn from the study due to rectal temperatures being 40.5°C or higher for four consecutive days.

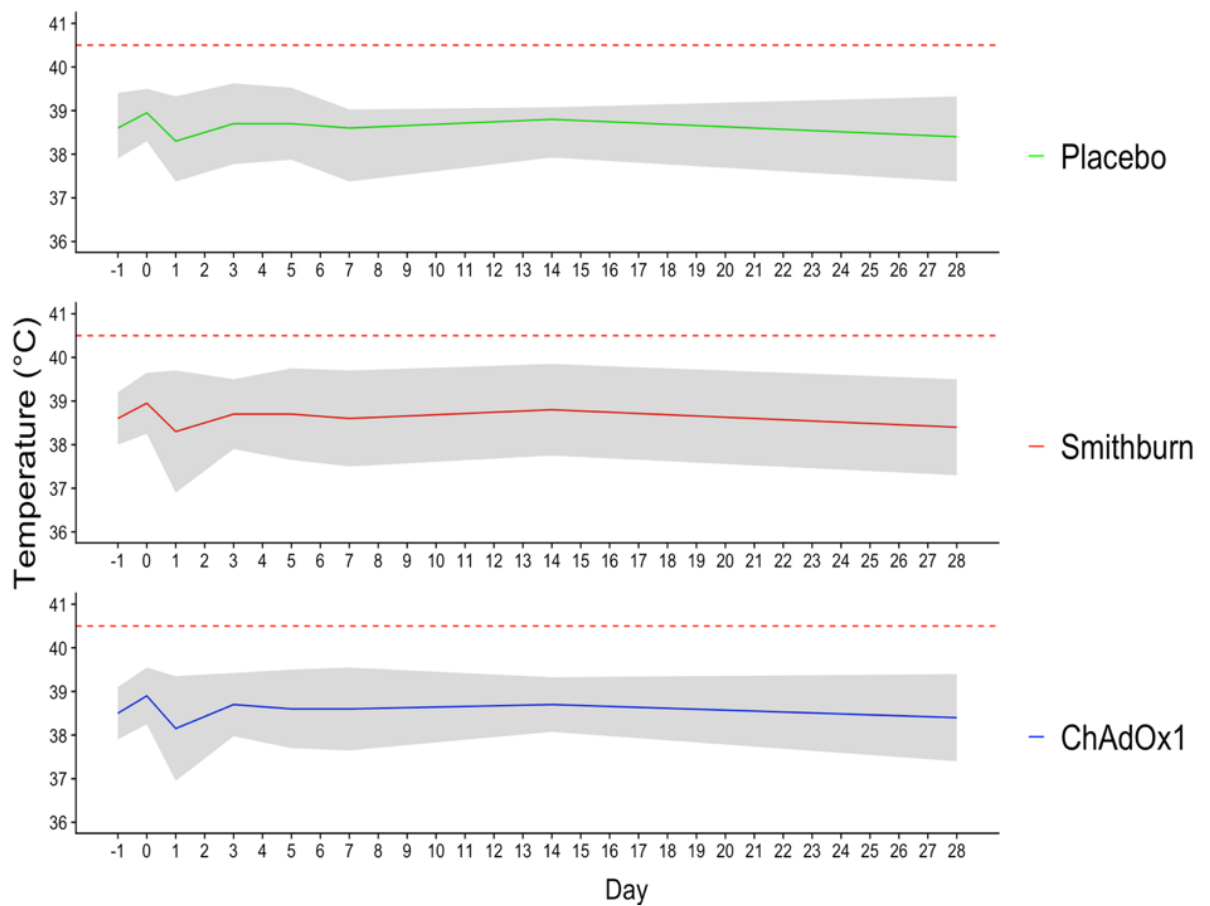


Figure 6.4 Rectal temperature (°C) for cattle over the 28-day post-vaccination period. The solid line is the median rectal temperature and the grey ribbon is the interquartile range. The red dotted line is the threshold at which animals would be removed from the study on the fourth consecutive day.

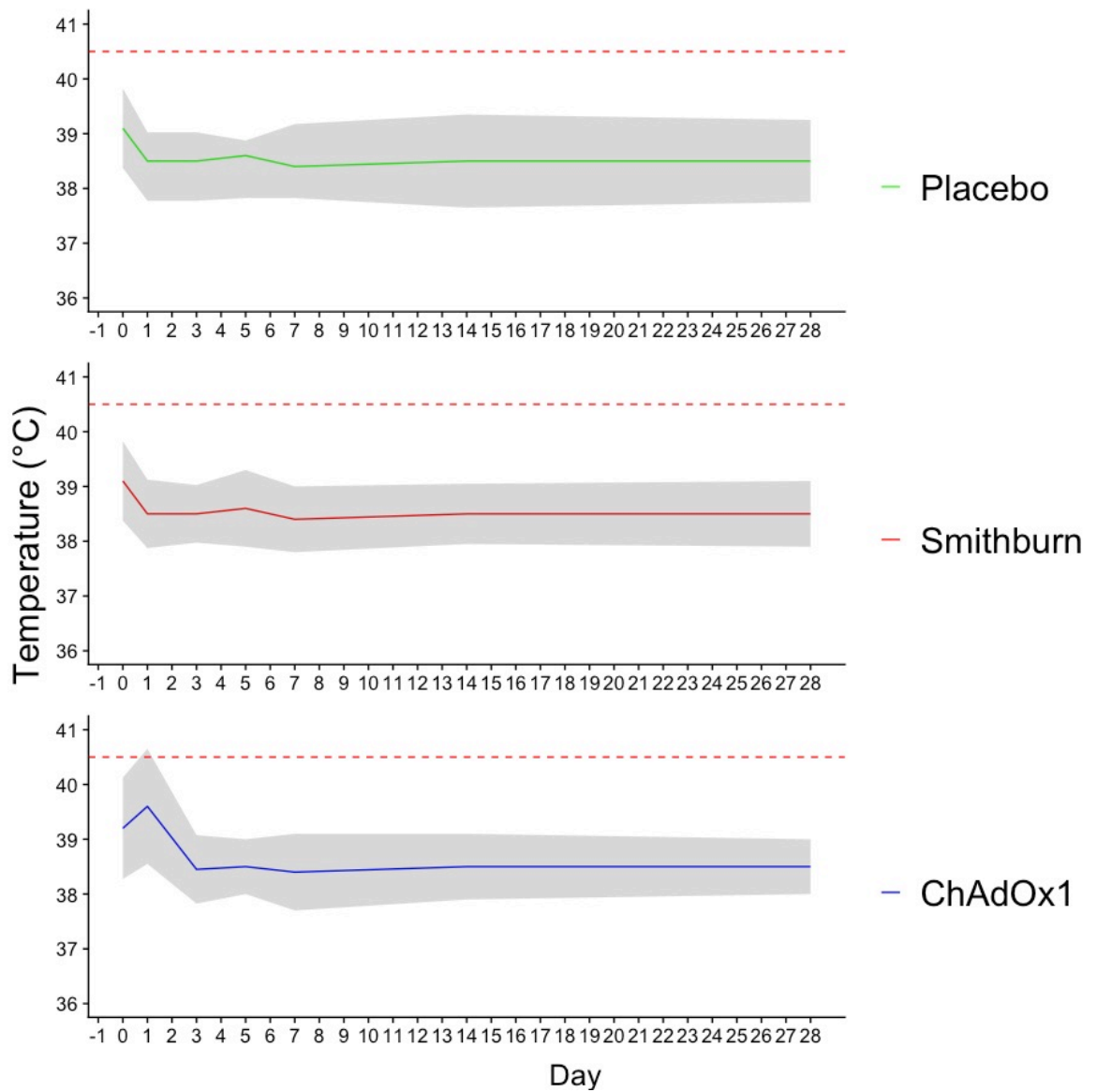


Figure 6.5 Rectal temperature (°C) for sheep over the 28-day post-vaccination period.

The solid line is the median rectal temperature and the grey ribbon is the interquartile range.

The red dotted line is the threshold at which animals would be removed from the study on the fourth consecutive day.

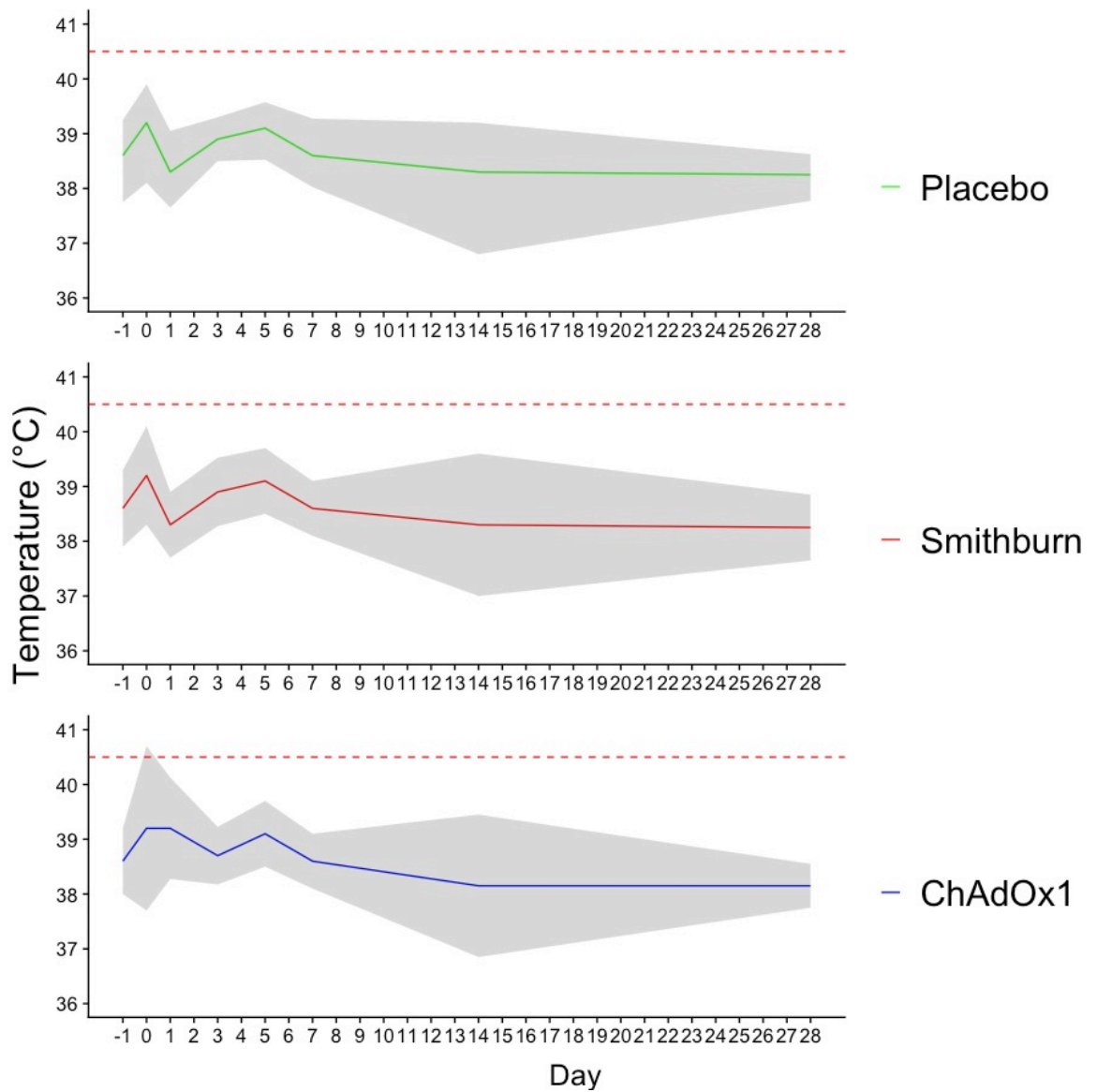


Figure 6.6 Rectal temperature (°C) for goats over the 28-day post-vaccination period. The solid line is the median rectal temperature and the grey ribbon is the interquartile range. The red dotted line is the threshold at which animals would be removed from the study on the fourth consecutive day.

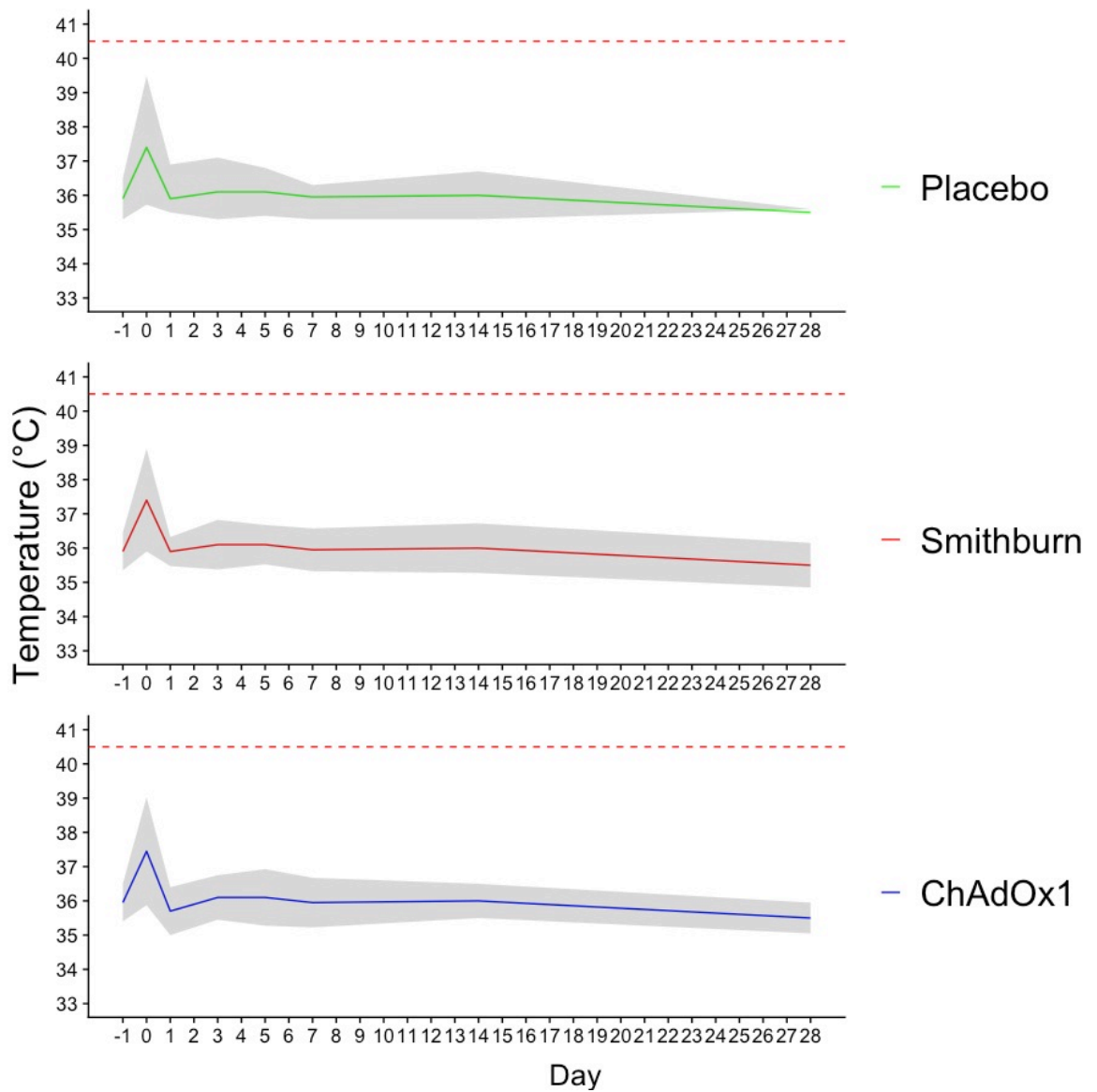


Figure 6.7 Rectal temperature (°C) for camels over the 28-day post-vaccination period. The solid line is the median rectal temperature and the grey ribbon is the interquartile range. The red dotted line is the threshold at which animals would be removed from the study on the fourth consecutive day.

6.2.3. Safety results: Clinical observations

A good safety profile was demonstrated in both vaccines for all species. Local and systemic adverse events are summarised in table 6.3. There were no changes in appetite, depression and respiratory distress during the 28-day period in any species. Isolated incidents in animals were reported for a number of adverse events (Table 6.5). A high number of camels, irrespective of treatment group, had mild loss of body condition. Similarly, a number of cattle, irrespective of treatment group and over different time points also had mild loss of body condition. There were no severe adverse events reported in goats, cattle or camels. One sheep had a severe nasal discharge event on Day 28, but no other severe adverse events were reported during the first 28 days post-vaccination in sheep.

The most frequent reports of adverse events were mild and moderate nasal discharge (Table 6.4). The majority of all sheep, goats and cattle displayed mild nasal discharge at least once. For goats, univariate analysis showed that there was a significant difference for the different vaccination groups and reporting of mild nasal discharge ($p=0.03$). In sheep and cattle there was also a significant difference between the frequency of moderate nasal discharge in treatment groups (univariate analysis $p=0.05$ and $p=0.08$ respectively).

Heatmaps illustrate the longitudinal reporting of nasal discharge events in individual goats for ChAdOx1 RVF and Smithburn vaccine groups (Figure 6.8 and 6.9). Mild nasal discharge was reported throughout the 28-day sampling time period. The frequency of goats with mild nasal discharge reduced by day 28 but remained constant for cattle and sheep (Figure 6.10).

A binomial generalised linear mixed model was constructed to assess the factors influencing nasal discharge in goats (Table 6.5). After adjusting for other variables there were no significant differences between vaccination groups. However, there were significantly fewer

reports of mild nasal discharge on Day 0 and Day 28 ($p<0.001$), in males compared to females ($p<0.001$) and in less heavy animals ($p<0.001$) (Table 6.5).

At the time of writing this thesis electronic records were available showing which specific veterinarians observed the clinical observations at each time point for sheep, but not other species. Reporting of nasal discharge in sheep was significantly associated with a specific veterinarian in univariate ($p<0.001$) and multivariate analysis ($p<0.001$) (Table 6.6).

Table 6.4 Number (%) of animals record as having at least one adverse event during the 28 day study period

Species	ChAdOx1	Smithburn	Placebo	p-value
Mild Loss of Body Condition				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	21 (26.3)	18 (22.5)	5 (25)	0.9
Camels	8 (11.0)	14 (19.2)	4 (44.4)	0.2
Moderate Loss of Body Condition				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	2 (6.3)	0 (0)	0.6
Severe Loss of Body Condition				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Mild Depression				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	1 (1.3)	0 (0)	1
Cattle	2 (2.5)	0 (0)	0 (0)	0.6
Camels	0 (0)	0 (0)	0 (0)	-
Moderate Depression				
Sheep	1 (1.3)	0 (0)	0 (0)	1
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Severe Depression				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Mild Respiratory Distress				
Sheep	0 (0)	0 (0)	1 (5)	1
Goats	1 (1.1)	0 (0)	0 (0)	1
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Moderate Respiratory Distress				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Severe Respiratory Distress				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-

Species	ChAdOx1	Smithburn	Placebo	p-value
Mild Nasal Discharge				
Sheep	59 (73.8)	58 (72.5)	16 (80)	0.9
Goats	70 (87.5)	78 (97.5)	19 (95)	0.03
Cattle	75 (93.8)	77 (96.3)	20 (100)	0.67
Camels	0 (0)	0 (0)	0 (0)	-
Moderate Nasal Discharge				
Sheep	15 (18.8)	17 (21.3)	0 (0)	0.05
Goats	9 (11.3)	3 (3.8)	1 (5)	0.2
Cattle	3 (3.8)	2 (2.5)	3 (15)	0.06
Camels	0 (0)	0 (0)	0 (0)	-
Severe Nasal Discharge				
Sheep	0 (0)	1	0 (0)	1
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Mild Cough				
Sheep	0 (0)	2 (2.5)	0 (0)	0.6
Goats	7 (8.8)	4 (5)	2 (2.5)	0.6
Cattle	1 (1.3)	0 (0)	0 (0)	1
Camels	0 (0)	0 (0)	0 (0)	-
Moderate Cough				
Sheep	1 (1.1)	0 (0)	0 (0)	1
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Severe Cough				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Mild Lameness				
Sheep	0 (0)	1 (1.3)	0 (0)	1
Goats	2 (2.5)	14 (17.5)	1 (5)	0.003
Cattle	0 (0)	1 (1.3)	0 (0)	1
Camels	1 (1.3)	1 (1.3)	0 (0)	1
Moderate Lameness				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	2 (2.5)	2 (2.5)	0 (0)	1
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	1 (1.3)	0 (0)	0 (0)	1
Severe Lameness				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Mild Loss of Appetite				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-

Species	ChAdOx1	Smithburn	Placebo	p-value
Moderate Loss of Appetite				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-
Severe Loss of Appetite				
Sheep	0 (0)	0 (0)	0 (0)	-
Goats	0 (0)	0 (0)	0 (0)	-
Cattle	0 (0)	0 (0)	0 (0)	-
Camels	0 (0)	0 (0)	0 (0)	-

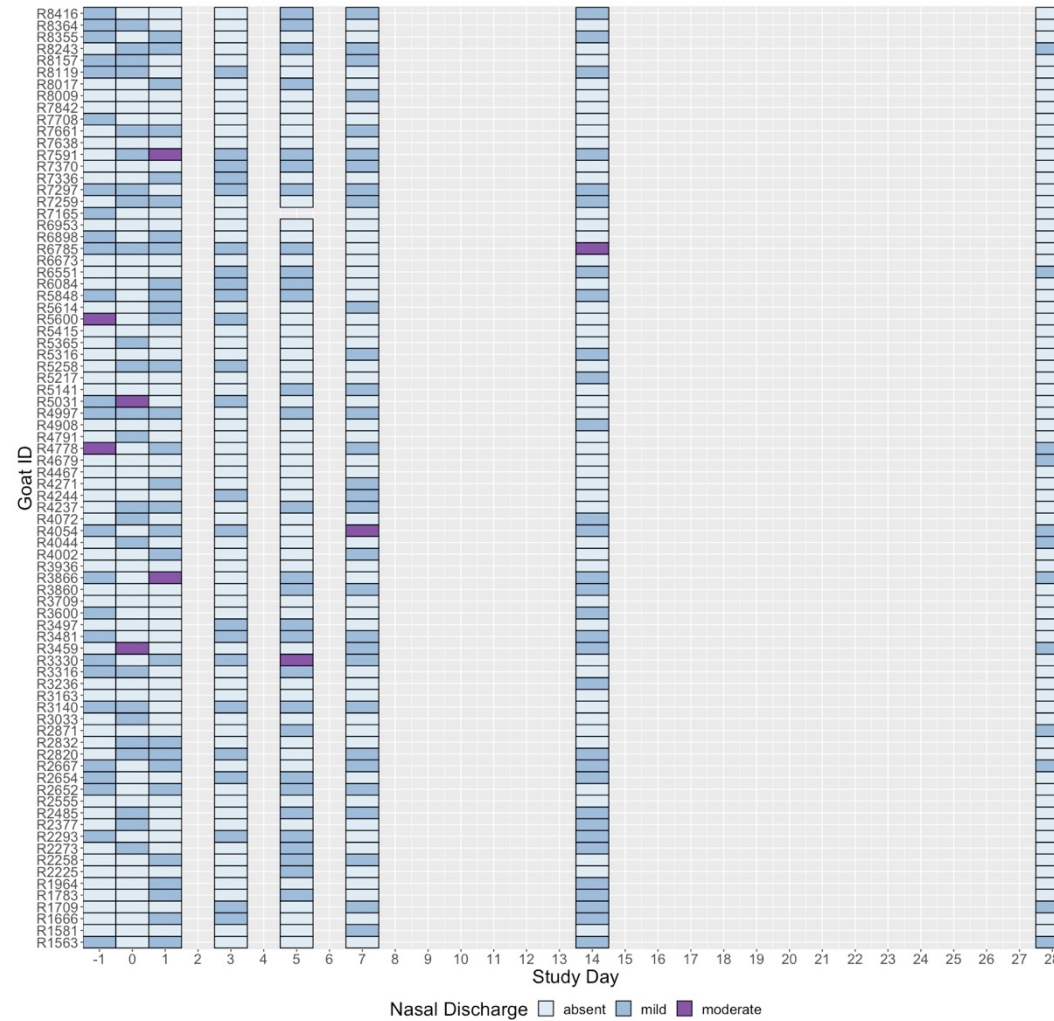


Figure 6.8 Reports of nasal discharge for each goat (Animal ID, y-axis) vaccinated with ChAdOx1 RVF shown by study day (x-axis).

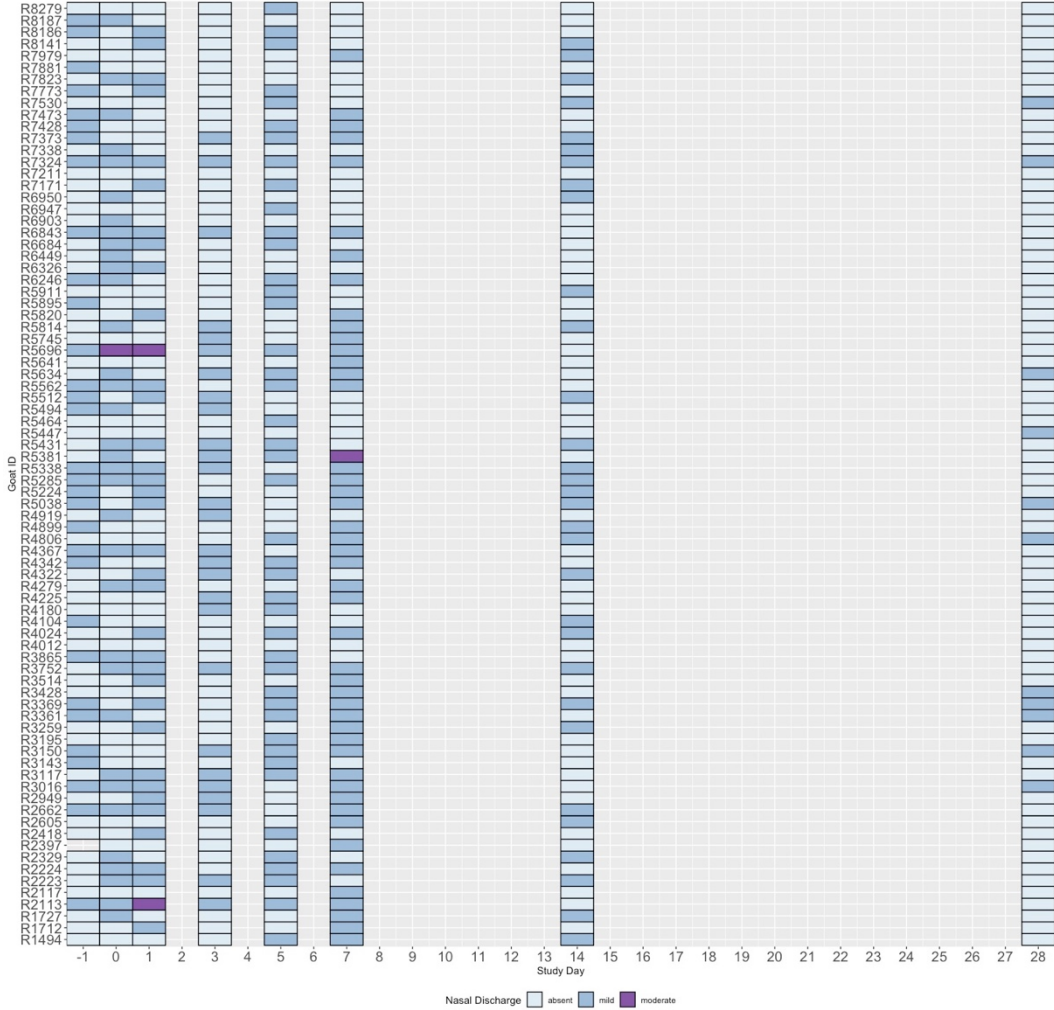


Figure 6.9 Reports of nasal discharge for each goat (Animal ID, y-axis) vaccinated with Smithburn shown by study day (x-axis).

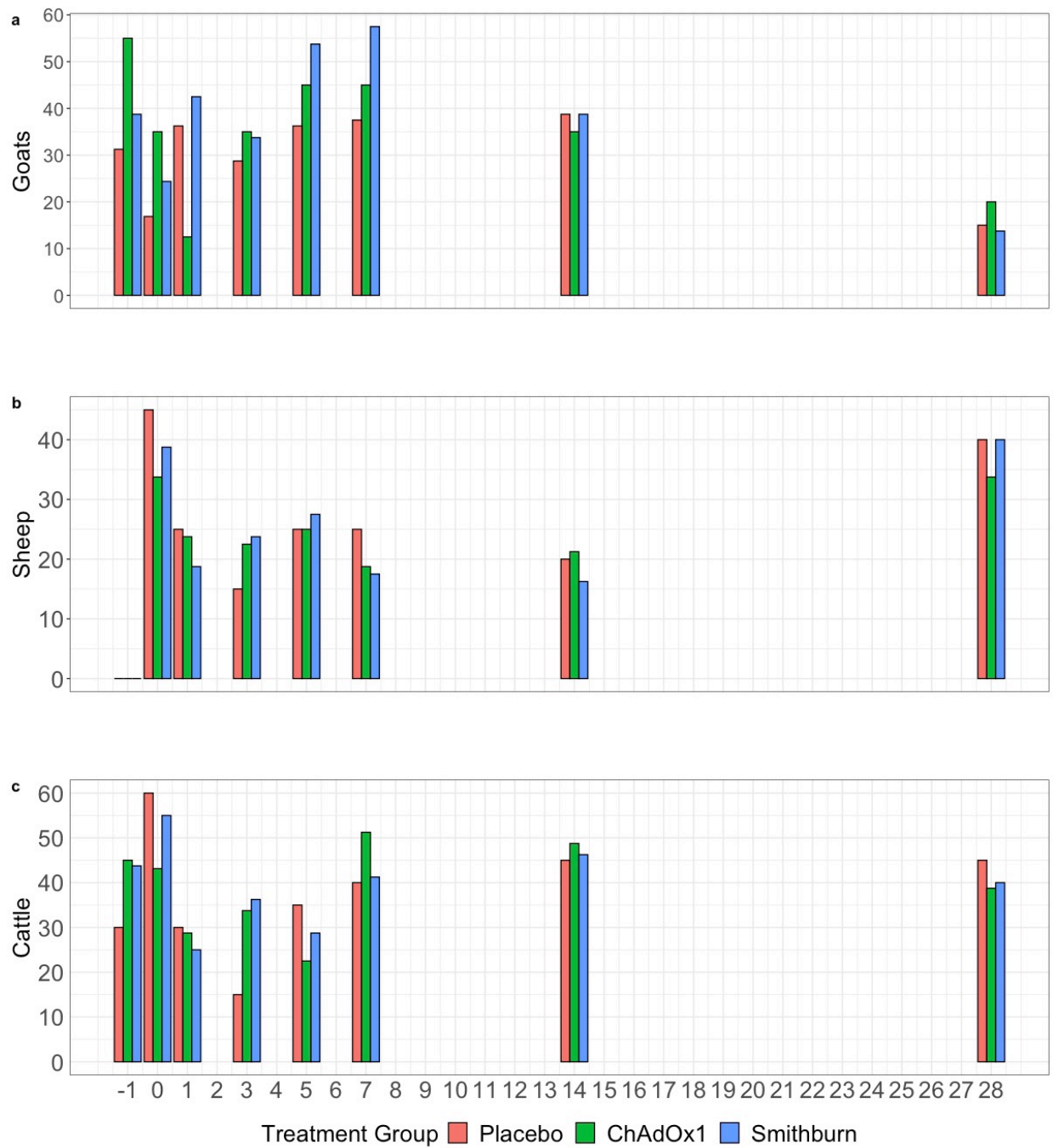


Figure 6.10 Proportion of animals by treatment group which were reported to have mild nasal discharge by study day.

Table 6.5 Binomial generalised linear mixed model result for nasal discharge in goats

Variable	Coefficient	Standard Error	p-value
Intercept	0.43	0.36	0.22
Vaccine group			
Placebo	Baseline	-	-
ChAdOx1 RVF	-0.17	0.24	0.48
Smithburn	0.16	0.24	0.50
Study Day			
Day -1	Baseline	-	-
Day 0	-2.0	0.31	<0.001
Day 1	0.08	0.22	0.74
Day 3	-0.33	0.23	0.16
Day 5	0.34	0.23	0.14
Day 7	0.45	0.23	0.05
Day 14	0.02	0.23	0.91
Day 28	-1.37	0.27	<0.001
Sex			
Male	Baseline	-	-
Female	0.62	0.16	<0.001
Weight (kg)	-0.07	0.02	0.001
Age	0	0	0.4

Table 6.6 Binomial generalised linear mixed model for nasal discharge in sheep

Variable	Coefficient	Standard Error	p-value
Intercept	-5.21	3.55	0.14
Veterinarian			
Vet 1	Baseline	-	-
Vet 2	1.06	0.18	<0.001
Vaccine Group			
Placebo	Baseline	-	-
ChAdOx1 RVF	-0.13	0.31	0.68
Smithburn	-0.05	0.31	0.88
Day			
Day 0	Baseline	-	-
Day 1	-0.92	0.25	<0.001
Day 3	1.15	0.26	<0.001
Day 5	1.59	0.27	<0.001
Day 7	0.82	0.25	<0.001
Day 14	0.88	0.25	<0.001
Day 28	1.38	0.21	<0.001
Sex			
Female	Baseline	-	-
Male	-0.15	0.22	0.48

Age

Age	0.01	0.01	0.36
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Mild lameness was reported for 17 goats and moderate lameness was reported in 4 goats.

Mild lameness was not sustained as 14 of these goats (82.4%) only reported lameness once.

Univariate analysis found that reporting mild lameness was significantly associated with the Smithburn vaccine group. When multivariate analysis was conducted no significant difference was reported (Table 6.7).

Table 6.7 Binomial generalised linear mixed model for lameness in goats

Variable	Coefficient	Standard Error	P value
Intercept	-8.31	7.29	0.25
Vaccine Group			
Placebo	Baseline	7.13	-
ChAdOx1 RVF	1.82	7.07	0.8
Smithburn	3.35	1.3	0.64
Study Day			
Day -1	Baseline	1.19	-
Day 0	0.759	1.22	0.56
Day 1	1.95	1.30	0.1
Day 3	1.45	0.0000005	0.22
Day 5	0.753	1.63	0.56
Day 7	-0.499	0.00646	1
Day 14	-0.467	1.30	0.77
Day 28	-0.177	0.164	1
Sex			
Female	Baseline	-	-
Male	0.390	1.30	0.76
Weight	-0.0894	0.164	0.59
Age	0.0006.05	0.0068	0.93

General Health Observations

A number of general health observation were recorded during the process of the trial. All of the goats suffered from ear infections; this was due to attaching the ear tags (no significant difference between treatment groups $p=0.7$). A total of 61/73 (83.6%) camels were diagnosed with alopecia and all camels had swollen cervical lymph nodes during the 28 days.

Mortalities

No mortalities were recorded for goats and post-mortem analysis for the individual deaths recorded for sheep, cattle and camels revealed mortalities were not associated with vaccination (Table 6.8).

Table 6.8 Field Trial Mortalities up to Day 28

Species	ChAdOx1	Smithburn	Placebo	Number sampled	Cause of death
Sheep	1 (1.25)	0 (0)	0 (0)	180	Oculovascular myiasis
Goats	0 (0)	0 (0)	0 (0)	180	-
Cattle	0 (0)	1 (1.25)	0 (0)	180	Backbone lumbar fracture
Camels	1 (1.25)	0 (0)	0 (0)	73	Unconfirmed, suspected trypanosomiasis

6.3. Conclusions

A comprehensive longitudinal safety analysis was conducted to assess the safety of the vaccines in the non-inferiority field trial. Both ChAdOx1 RVF and Smithburn vaccines were safe and well tolerated in all four livestock species tested. Safety results for the animals vaccinated with the ChAdOx1 RVF vaccine are in agreement with previous studies undertaken in experimental settings (Warimwe et al. 2016, Stedman et al. 2019).

There were no vaccine-related mortalities among the study animals. Due to the number of animals recruited to the trial, and it being conducted in the field, deaths due to other factors occurred. During the trial five sheep became infected with oculovascular myiasis (caused by larvae of sheep botfly, *Oestrus ovis*, Figure 6.11). Animals were treated with ivermectin, but, unfortunately, one sheep succumbed to the encephalitic form of the disease. One heifer unfortunately died due to a backbone lumbar fracture. She was treated with anti-inflammatories and observed for several days, but had to be euthanised for welfare reasons. Diagnosis of the camel death was unconfirmed but a suspected diagnosis of trypanosomiasis was recorded. Due to the non-specific clinical signs and low parasitaemia, it can be difficult to diagnose trypanosomiasis in camels (Gadahi et al. 2013)

The injection site reactions reported in the small number of goats, one cow and one camel are likely due to individual host immune responses for those particular animals. A large proportion of sheep did have injection site reactions, specifically hardening of the area. However, there was no significant difference in the frequency of reactions between treatment groups.



Figure 6.11 Botfly larvae causing mortality to sheep R1476

Rectal temperatures of study animals remained within normal levels throughout the study. No animals had to be withdrawn from the study because they had a rectal temperature over 40.5°C for the fourth consecutive day. The peak temperature values recorded in sheep, goats and cattle, which occurred during the first several days of sampling, may be a product of a new environment: the stress of handling and sampling, which a majority of animals get used to after a few days. Rectal temperature must be analysed with caution as a number of factors can affect recordings. Body temperature of animals can easily fluctuate throughout the day and thus sampling at different times of the day can affect this. In addition, behaviour of the animals (e.g. if they are stressed by sampling), age and weight of animal and how animals are kept can all influence rectal temperature.

Adverse events were predominantly mild across the different vaccine groups and adverse events reported were not associated with the type of vaccine administered. The clinical observations have shown a degree of subjectivity in the scoring, specifically one veterinarian was more likely to report mild nasal discharge in sheep than the other field vet in the study. At the time of writing this thesis the datasets did not include the records of the reporting veterinarian for clinical observations in goats, cattle and camels. This emphasises how scoring can be subjective. Further to this mild nasal discharge was reported in some animals at day -1 (baseline) before vaccination further suggesting this may not vaccine induced.

Poor body condition was recorded for a number of camels and cattle during the course of the study. The likely diagnosis for the poor body conditions in the camels was mange. Camels were treated with ivermectin to alleviate symptoms but in the 28-day post-vaccination period no change in condition was reported. It was recognised early in the process of vaccinating and sampling the cattle that more time was required to complete sampling these animals. This meant the study animals were confined to the paddock for a substantial amount of time

before they were allowed to graze which could explain the loss of body condition. This was recognised within a few days and hay supplementation was provided in the paddocks soon after this which reduced reporting of loss of body condition.

A number of limitations have been raised with regards to the field trial and investigating the safety of the vaccines. It has previously been acknowledged that there is a difficulty in establishing a valid and repeatable measure of clinical outcomes in veterinary clinical trials (Friese et al. 2013). Although there was a defined case definition and scoring guide for the clinical assessments, observations such as nasal discharge do have an element of subjectivity from the veterinarian reporting. Bias was fractionally mitigated as animals were sampled in random order at every sampling time point, so each vet would not necessarily examine the same animal at every time point. However, there were only two veterinarians sampling which does limit the bias mitigation.

For clinical trials all safety records need to be completed on hard paper copies. In the future using an electronic system (such as Epicollect or RedCap) in the field in conjunction with the hard copies would be useful for checking any adverse clinical signs that may need to be monitored in real time. This would be considered a duplication of effort, so a recommendation would be to have more communication with regulators to understand the rationale of having hard copies of data collection forms and understanding if this is needed if other regulated electronic systems could be put in place.

This chapter has discussed safety of the vaccine in animals only. The chimpanzee adenovirus used in the vaccine is replication deficient and so cannot propagate (Dicks et al. 2012). As a GM vaccine the regulators required evidence to show that there was no shedding of the chimpanzee adenovirus in the environment and also the food chain. PCR results undertaken

on blood, nasal and rectal samples of the animals' post vaccination show no adenovirus was shed. This work was undertaken by my colleague James Nyagwange at the KEMRI-Wellcome Trust Programme (unpublished).

There is very little in the literature on safety reporting and analysis for veterinary vaccines. The most comparable field trial conducted with an RVF vaccine was the randomized controlled field trial assessing the Clone 13 vaccine in livestock in multiple farms in Kenya (Njenga et al. 2015). Reporting of safety and clinical assessment was limited, and the only details provided are that rectal temperatures were assessed at day 1, 2 and 3 post-injection and no fever was reported.

A risk/benefit analysis is typically conducted to assess whether a vaccine is accepted within the market depending on recorded adverse reactions. Mild and moderate adverse events are often tolerated provided there is no significant suffering or long-term damage (Francis, M. personal communication Nov 2019). Results from this trial suggest the vaccine would be marketable as there were limited safety adverse events reported and these adverse events were not significantly more frequent in the ChAdOx1 RVF vaccine group.

7. A non-inferiority Rift Valley fever vaccine field trial to assess vaccine immunogenicity in multiple livestock species in Kenya: Vaccine immunogenicity

7.1. Background

The immunogenicity of ChAdOx1 RVF and Smithburn vaccines are assessed in this chapter. Since RVFV neutralising antibodies are the main correlate of protection from RVF, the primary endpoint for this analysis was the proportion of animals mounting a neutralising antibody response as measured 28 days post-vaccination. Non-inferiority comparisons for each species were made between the ChAdOx1 RVF and Smithburn vaccine groups as described in detail in chapter 5. An interim analysis is presented in this thesis, with final analysis and results expected once all trial data are collected and trial database locked.

Since RVFV can transmit between mosquitoes, different livestock species and wildlife over large areas, being able to differentiate between infected and vaccinated animals is important especially in the context of pastoralist or transhumance farming. The Smithburn and inactivated commercialised vaccines are based on whole RVFV and thus produce a similar antibody profile as that elicited by natural RVFV infections. Therefore, diagnostic tests are unable to differentiate between infected animals and those that have been vaccinated (DIVA). This can have large implications on large-scale RVF surveillance particularly as it is not well known how efficacious the current commercialised vaccines are in large populations. As the ChAdOx1 RVF vaccine works by encoding the RVF Gn and Gc glycoproteins only, it has the characteristics to be DIVA compliant (i.e. serological kits based on RVFV components absent in ChAdOx1 RVF would test negative for ChAdOx1 RVF vaccinated animals, but positive for RVFV-infected animals). Small-scale studies have already confirmed this for the ChAdOx1 RVF vaccine but have yet to directly compare responses to animals vaccinated with the Smithburn vaccine or with a larger population in the field (Stedman et al. 2019). Therefore, this chapter also reports antibody immunogenicity results based on an ELISA diagnostic test to distinguish whether ChAdOx1 RVF vaccine is a DIVA compliant vaccine.

7.2. Methods

The rationale and design of the non-inferiority trial can be found in chapter 5 of this thesis. The CONSORT checklist for non-inferiority tests can be found in appendix A5.2. Briefly, sheep (Red Masaai-Dorper cross), goats (Galla breed), cattle (Boran breed) and dromedary camels were screened for previous exposure to RVFV using the ID Screen® RVF kit as per manufacturer's recommendations. Only seronegative animals were enrolled to the trial (n=180 for sheep, goats and cattle, n=73 at the time of thesis submission for camels) and randomly assigned to a treatment group where they either received ChAdOx1 RVF, Smithburn or placebo vaccination (see Table 5.1).

7.2.1. DIVA assessment

The ID Screen® RVF ELISA kit was used to assess DIVA compatibility in study animals. This test was used on all samples from day -1 (pre-vaccination) and day 28 (post-vaccination). A detailed description of the test methodology can be found in section 3.2.3.

7.2.2. Focus Reduction Neutralisation Test (FRNT)

Neutralising antibody responses were assessed using a focus reduction neutralising antibody test (FRNT₈₀) at the KEMRI-Wellcome Trust Programme by my colleague John Gitonga. For this thesis, an interim analysis was carried out on day 28 FRNT₈₀ data. RVF virus infects cells causing cytopathology that can be visualised as foci on immunostaining the virions in infected cells. In this interim analysis, an animal was deemed to have seroconverted if pre-incubation of its serum (collected at day 28) with RVFV led to an 80% (FRNT₈₀) reduction of virus infectivity relative to virus incubated in the absence of sera. To perform the assay, vero cells were seeded by adding 1 million cells per plate onto 96 well tissue culture plates and incubated overnight in a CO₂ incubator to attain 90% confluency. The serum samples were heat-inactivated at 56°C for 30 minutes before use. Heat-inactivated samples were

diluted to 1:50 with D10 media. Dilutions were run in duplicate. Diluted serum was transferred to a 96-well round bottom plate. Each plate also contained virus and media only wells. RVF virus (MP-12 strain) was diluted to 1×10^3 focus-forming units (FFU) in D10 media which was then transferred to each well (50 μ L) containing 50 μ L of the diluted serum making the dilution 1:100. This mixture was incubated in a CO₂ incubator for one hour at 37°C after which 100 μ L of the virus-serum mixture was transferred to the Vero cells and incubated for 2 hours in the CO₂ incubator at 37°C. During these 2 hours the plates were rocked every 15 minutes to allow uniform distribution of the virus on the Vero cells. Using a micropipette, the virus-serum mixture was carefully aspirated from the wells without disrupting the monolayer. D10 media was then added to the wells (100 μ L) and incubated for 24 hours at 37°C with 5% CO₂. The D10 media was then aspirated from the wells, followed by gentle washing with 200 μ L of 1xPBS, again without disrupting the monolayer. Ice cold 4% Paraformaldehyde (PFA) (100 μ L per well) was added to the plates and incubated for 15 minutes at room temperature to fix the cells. The plates were washed 3 times with 200 μ L of 0.5% Triton X-100 in PBS. The permeabilization buffer (0.5% Triton X-100 in PBS) was added to the wells (100 μ L) and incubated for 20 minutes at room temperature. The permeabilization buffer was flicked off and blocking buffer (100 μ L of casein) was added. Plates were incubated at room temperature for 30 minutes, then washed 3 times with the permeabilization buffer (200 μ L per well). The 4D4 mouse anti-RVFPV Gn monoclonal antibody was diluted in permeabilization buffer (0.5 μ g/ml, 50 μ L per well) and added to the plates which were incubated for 2 hours at 37°C. Plates were washed 3 times with 200 μ L of permeabilization buffer per well. The secondary HRP-conjugated anti-mouse antibody was then added (1:1000 diluted in permeabilization buffer, 50 μ L) and incubated for 1 hour at 37°C. Wells were washed four times with 200 μ L of permeabilization buffer per well and the 1X DAB substrate (0.5ml 10X DAB concentrate and 4.5ml Hydrogen Peroxide Buffer) was added to the wells (100 μ L). Plates were incubated in the dark for 5-10 minutes checking

occasionally for colour development. Once foci were clearly visible by eye (a red/brown colour), plates were washed with gentle running tap water to remove the substrate. They were then left to dry overnight, and foci were counted using an AID ELISPOT reader. Seroconversion was defined as an 80% reduction of RVFV infectivity of Vero cells relative to cells incubated with RVFV in the absence of any sera, henceforth termed FRNT₈₀.

7.2.3. Statistical methods

Fisher's test was conducted to assess if there was a significant difference in RVF IgG antibody seroconversion by treatment groups for DIVA assessment. Shapiro-Wilk normality test was used to assess normality of data. Binomial family generalised linear models were used to assess differences in seroconversion of neutralising antibodies between treatment groups, accounting for weight, age and sex. The protocol for the non-inferiority sample sizes and non-inferiority margin can be found in chapter 5. Briefly, a 10% non-inferiority margin was used, assuming 97% prevalence of seroconversion in all species (based on results for the Clone 13 RVF vaccine as there is no published seroconversion rates for Smithburn vaccine) and a two-sided alpha of 5%. Non-inferiority analysis was calculated using the `confinter` package in the R statistical environment (version 3.3.3) (R Core Team 2013). This analysis used confidence intervals generated from the linear regression models, comparing neutralising antibody seroconversion in ChAdOx1 RVF vaccinated animals to those vaccinated with the Smithburn vaccine (Appendix A7.1 Non-inferiority analysis code). The null hypothesis was therefore based on the lower bounds of the 95% confidence intervals of the difference between the ChAdOx1 RVF and Smithburn vaccine not being smaller than -10%. The alternative hypothesis was that the lower bounds of the 95% confidence intervals of the difference between ChAdOx1 RVF and Smithburn vaccine was greater than -10%.

7.3. Results

7.3.1. DIVA compatibility of ChAdOx1 RVF

No sheep, cattle or camels vaccinated with the ChAdOx1 RVF vaccine or placebo vaccine seroconverted, suggesting the ChAdOx1 RVF vaccine is DIVA compliant (Table 7.1). The only animals that seroconverted were those vaccinated with the Smithburn vaccine ($p < 0.001$). However not all animals vaccinated with the Smithburn vaccine seroconverted and the proportion of those that did varied by species (Table 7.1). Smithburn vaccinated goats had the highest seroconversion to the DIVA kit, followed by camels, sheep and cattle (Table 7.1).

Table 7.1 Percentage of species undergone RVF IgG seroconversion by treatment group

Treatment Group	Species	RVF IgG Seroconversion (%)	Lower CI (%)	Upper CI (%)
ChAdOx1 RVF	Sheep	0 (0/79)	0	5.8
	Goats [□]	0 (0/79)	0	5.8
	Cattle	0 (0/80)	0	5.7
	Camels	0 (0/31)	0	13.7
Smithburn	Sheep	16.3 (13/80)	11.1	31.2
	Goats	40 (32/80)	29.4	51.6
	Cattle	3.2 (2/79)	0.04	9.7
	Camels	31.3 (10/32)	16.7	50.1
Placebo	Sheep	0 (0/20)	0	2.0
	Goats	0 (0/20)	0	2.0
	Cattle	0 (0/20)	0	2.0
	Camels	0 (0/9)	0	37.1

[□]One goat vaccinated with placebo vaccine tested positive for RVF IgG at day -1 and so was removed from analysis in this table.

7.3.2. Induction of RVFV neutralising antibodies

The ChAdOx1 RVF vaccine elicited RVFV neutralising antibodies in all species. Table 7.2 shows the percentages of animals that seroconverted in the different vaccination groups. Neutralising antibody seroconversion was highest in sheep (94.9%, 95% CI 88.0, 98.1) and goats (97.5%, 95% CI 91.5, 99.4) vaccinated with ChAdOx1 RVF. The number of animals that seroconverted having been vaccinated with the ChAdOx1 RVF vaccine was significantly higher for sheep ($p > 0.001$), goats ($p > 0.001$) and cattle ($p > 0.001$) compared to those vaccinated in the Smithburn vaccine or the placebo group (Table 7.2). There was very poor seroconversion among Smithburn vaccinated animals, especially in cattle where only 3.8%, (95% CI 1.2, 10.2) of animals seroconverted as measured by FRNT₈₀. Three of the 20 goats in the placebo seroconverted, despite having no response on the DIVA ELISA which would detect responses following RVF exposure if this had occurred on the farm. None of the placebo controls in any of the other species seroconverted either by FRNT₈₀ or the DIVA ELISA.

As part of the trial design, all vaccine/placebo groups were matched by sex, age and body weight; seroconversion did not differ by any of these variables in sheep, goats or cattle ($p > 0.05$ for all) (appendix A7.2). In camels, male camels were more likely to seroconvert than female camels ($p < 0.001$) but enrolment and vaccinations are still ongoing.

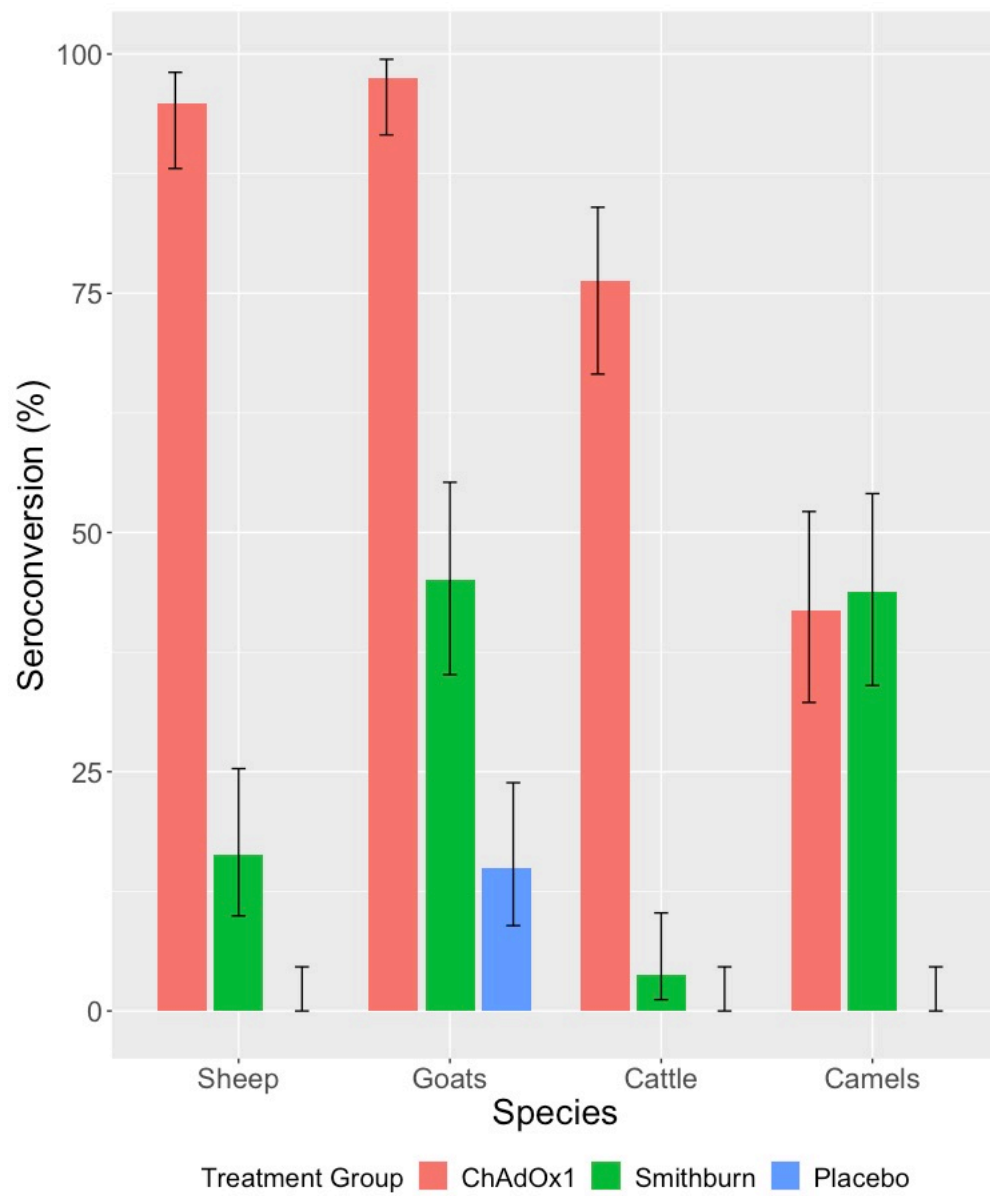


Figure 7.1 Seroconversion of neutralising antibodies to RVF by treatment group in sheep, goats, cattle and camels. Error bars indicate the confidence intervals.

Table 7.2 RVF neutralising antibody seroconversion by vaccine group and species

Species	Treatment Group	FRNT ₈₀ Seroconversion (%)	Lower CI (%)	Upper CI (%)
Sheep	ChAdOx1 RVF	94.9 (75/79)	86.9	98.4
	Smithburn	16.3 (13/80)	9.3	26.6
	Placebo	0 (0/20)	0	2
Goats	ChAdOx1 RVF	97.5 (78/80)	90.4	99.6
	Smithburn	45 (36/80)	34	56.5
	Placebo	15 (3/20)	4	38.9
Cattle	ChAdOx1 RVF	76.3 (61/80)	65.2	84.8
	Smithburn	3.8 (3/79)	1.2	10.2
	Placebo	0 (0/20)	0	2
Camels	ChAdOx1 RVF	41.9 (13/31)	25.1	60.7
	Smithburn	43.8 (14/32)	26.8	62.1
	Placebo	0 (0/9)	0	37

7.3.3. Non-inferiority analysis

At 28 days post-vaccination ChAdOx1 RVF vaccine was non-inferior for eliciting neutralising antibodies to RVF in sheep, goats, cattle and camels and the null hypothesis (the difference in the lower bounds of the 95% confidence intervals is less than -10%) was rejected (Figure 7.2). The alternative hypothesis: the lower bounds of the 95% confidence intervals is more than -10% is accepted. For camels, the results were not as evident compared to the other species (Figure 7.2).

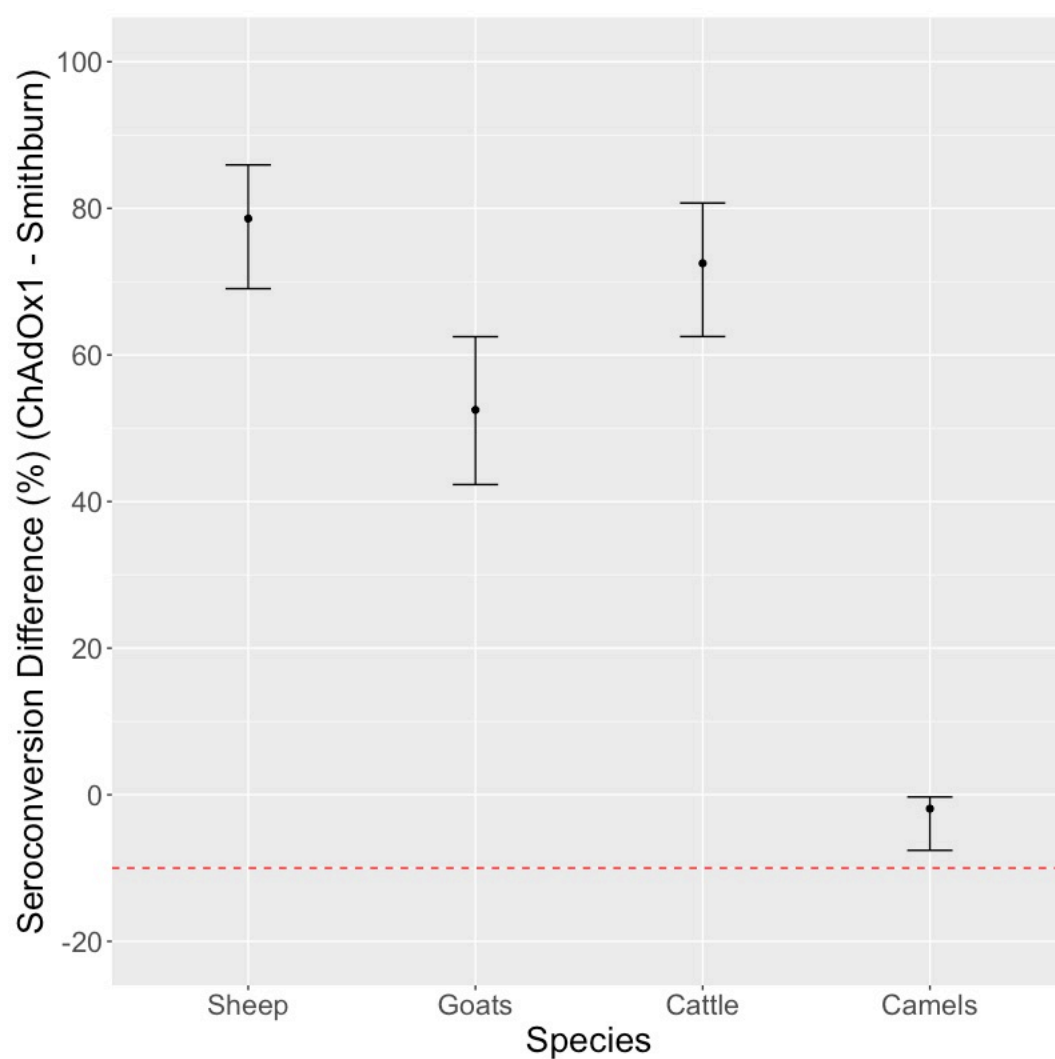


Figure 7.2 Forest plot illustrating non-inferiority analysis of ChAdOx1 RVF vaccine: RVF Seroconversion difference between ChAdOx1 RVF and Smithburn vaccine. Red dotted line indicates the 10% non-inferiority margin. Error bars indicate confidence intervals.

7.3.4. FRNT₈₀ and ID Screen® ELISA test for agreement

In this analysis two tests were used to analyse immune response to the Smithburn vaccine. A kappa test was conducted to assess test agreement between the ID Screen® ELISA and the FRNT₈₀ for reporting seroconversion in the Smithburn group. The kappa coefficient ranged from 0.31 for testing camels, 0.81 for sheep, 0.79 for cattle and 0.85 for goats (Appendix A7.3). Overall the kappa coefficient was high suggesting that there is a high level of agreement between the tests. Table 7.3 shows a cross tabulation table of the ID Screen® ELISA and the FRNT₈₀ for reporting seroconversion in the Smithburn group with all species pooled. Results show high sensitivity at detecting Smithburn seropositive cases when compared with the FRNT₈₀ results and a high specificity at detecting Smithburn seronegative cases when compared with FRNT₈₀ results (for species specific results see appendix A7.3). When pooling the species, the sensitivity was reported as 84% and specificity as 95% and the Kappa coefficient was 0.79.

Table 7.3 Number of IgG and neutralising antibody seroconversions in pooled species from the Smithburn group using the ID Screen® ELISA and the FRNT

Pooled Species ID Vet ELISA	FRNT ₈₀			
		Positive	Negative	Total
	Positive	54	11	65
	Negative	10	194	204
	Total	64	205	269

7.4. Conclusions

The results from this chapter show that ChAdOx1 RVF is a suitable veterinary vaccine to be taken forward for registration and commercialisation. The non-inferiority assessment concluded that the ChAdOx1 RVF vaccine was non-inferior to the Smithburn vaccine for sheep, goats, cattle and camels. As explained in chapter 5, this thesis has been written before the completion of the camel study and hence not all the camels required for the non-inferiority sample size have been tested. Camel results presented in this chapter are therefore not final. The smaller difference between ChAdOx1 RVF and Smithburn in camels may be due to the incomplete sampling, but it is also worth noting that manufacturers do not provide a set dose for the Smithburn vaccine for these animals. When writing the protocol for this study the field trial team consulted the vaccine manufacturers who suggested using the same dose as would be used for cattle (which is what was done). As a target species for RVF vaccination, camels should be included in quality control testing and proper dosing advised.

The results also confirmed that ChAdOx1 RVF is a DIVA compliant vaccine for all livestock species and with seroconversion rates suggesting it may be superior to the Smithburn vaccine. Along with the additional desirable safety characteristics for pregnant sheep and goats (Stedman et al. 2019), use in outbreaks and the ease of manufacture compared to the Smithburn vaccine, ChAdOx1 RVF has clear potential as a veterinary vaccine against RVF.

Study results have revealed the low immunogenicity of the Smithburn vaccine in multiple livestock species based on the FRNT and the neutralising antibody responses elicited as well as IgG responses from the ID Screen® ELISA. Other laboratory groups testing the Smithburn vaccine in the same indigenous livestock animals have observed similar responses and issues using the commercial ELISA kit (Personal communication, Dr Annie

Cook, July 2019). It is well known that cattle mount a weaker immune response to the Smithburn vaccine (Barnard 1979). Results from a complementary Smithburn vaccine study using the same batch of vaccine and the same FRNT and ELISA protocol on all four livestock species (n=5 per species) again confirmed the low immunogenicity for the Smithburn vaccine (unpublished pre-trial results).

The results from the FRNT and ELISA in the Smithburn vaccinated animals are not completely consistent although there is a high agreement between the two tests. It has previously been reported that the ID Screen® ELISA has high sensitivity (91-100%) and specificity (100%) (Kortekaas et al. 2012). This trial was conducted on a small number of cattle and sheep, it did not assess sensitivity or specificity of the ELISA in goats or camels and it did not state what breeds of animal were used. The results from this non-inferiority trial suggest the specificity of the ID Screen® kit is similar, although the sensitivity is lower than what is reported in the Kortekaas et al. (2012) paper. It must be acknowledged that without a gold standard method sensitivity and specificity results should be treated cautiously. It is worth noting that animals were recruited to the trial based on being seronegative to the ID Screen® ELISA. This has not impacted the results of the non-inferiority assessment although one goat vaccinated with placebo was reported as seropositive at day 28 on the commercial ELISA.

An alternative explanation for the low seroconversion rate for the Smithburn vaccine may be that the vaccine is not very immunogenic. Two batches of the Smithburn vaccine were used for the study, batch 10/2018 was used for the sheep and goats, batch 01/2019 was used for the cattle and camels. This still does not explain why the neutralising antibody response was so low in sheep, yet the majority of the goats seroconverted despite being vaccinated

with the same batch of vaccine. To address this further communication with the manufacturer is warranted to identify how quality control is conducted.

At the time of writing this thesis the study was still ongoing and not all the data collection forms had been uploaded. This included the data collection forms which recorded the individual breeds of sheep. The study used Masaai and Dorper-Masaai crosses. Masaai sheep are known to be resistant to some parasitic infections (Mugambi et al. 1997), and future research should analyse the results by the sheep breeds. The difference in seroconversion between female and male goats requires further research.

The FRNT₈₀ assay was used as it meant a greater number of samples could be tested at one time compared to Plaque Reduction Neutralisation Tests (PRNTs). There is no gold standard or reference method for RVF neutralisation assays. FRNT₈₀ was chosen due to the specificity of the assay and PRNT₈₀ tests are often used when assessing human neutralising antibodies to RVF (Pittman et al. 2016). Multi-channel pipettes make the FRNT an easier test to conduct, consuming less sample material and reagents and the final stages of fixing and staining are faster (Vaidya et al. 2010). At the time of writing this thesis, the trial and optimisation of assays, including the FRNT were still ongoing. Once the trial is complete, datasets can be locked, and definitive analysis can be conducted. This has clearly shown the need for standardised serological methods in the RVF field.

A significant limitation in RVF vaccinology is identifying correlates of protection and specifically what neutralising antibody titre is required for protection. There was no evidence of natural clinical RVF infection (symptoms) on the farm over the study period so protection against infection could not be assessed, although 3 of 20 goats in the placebo group developed RVFV neutralising antibody responses.

Smithburn vaccine was chosen as the comparator vaccine as it is the most widely used vaccine for RVF. Another RVF vaccine, the attenuated Clone 13 vaccine has also been tested in Kenya in large populations of cattle, sheep and goats. Neutralising antibody responses were high for the vaccine (97% in goats, 91% sheep and 67% cattle seroconverted). However, the results from this study show neutralising antibody responses are still higher for ChAdOx1 RVF in all species and furthermore ChAdOx1 RVF does not have issues with foetal malformations and stillbirths which has been reported with the Clone 13 vaccine (Makoschey et al. 2016, Stedman et al. 2019). It has previously been reported that cattle mount a weaker immune response when vaccinated with other RVF vaccines compared to other livestock species (Barnard et al. 1979, Njenga et al. 2015). Vaccinating cattle is likely to be important, because of the major role in the transmission dynamics of the virus (Gachohi et al. 2018, see chapter 8).

Reviews have commented on the transparency of non-inferiority study design (Frieze et al. 2013, Rehal et al. 2016). For this study a CONSORT statement checklist was completed to help mitigate this. The trial assumed a pragmatic *a priori* non-inferiority margin of 10%. The margin was chosen based on the consequences that low levels of vaccination could have on animal and human health, because little research has assessed seroconversion to the Smithburn vaccine in the field. If more research was available for the Smithburn vaccine efficacy in the field compared with a placebo, this could have helped determine a more suitable non-inferiority margin as the magnitude of benefit would be known. This is a general issue in veterinary medicine. Few placebo-controlled studies are conducted in the field, study designs are not standardised, and clinical endpoints are highly variable (Freise et al. 2013). A 10% margin is most commonly used in non-inferiority studies and has been recommended

in previous guidelines set out by regulatory offices, although neither the EMA nor FDA provide exact non-inferiority margins for vaccines (Donken et al 2015).

Although the study has confirmed the scientific rationale for registration of the vaccine, there is a limited market due to 1) the context that RVFV outbreaks have only been reported in the African continent and Saudi Arabia; 2) outbreaks are sporadic and IEPs can be as long or longer than a small ruminant's average lifespan; and 3) the unspecific clinical nature of the disease: farmers may not necessarily relate an infection to RVF but to other veterinary diseases such as brucellosis or Schmallenberg virus. This means willingness to pay for a RVF vaccine may be low. In the case of three Kenyan counties, cattle farmers did show willingness to pay (Wanyoike et al. 2019). Conversely the ChAdOx1 RVF vaccine could easily be manufactured quickly. The vaccine could be stockpiled by governments and only used when outbreaks are predicted or at the first instance an outbreak starts.

In conclusion, preliminary analysis of the immunogenicity and non-inferiority trial support the rationale for registering and commercialising the ChAdOx1 RVF vaccine for veterinary use in sheep, goats, cattle and camels in Kenya. Further investigation into the immunogenicity of the Smithburn vaccine is necessary as it is still currently the first choice for vaccination control strategies. Results from this study also support continued development for ChAdOx1 RVF into medical use for human clinical trials

8. Modelling the effect of vaccination on RVFV transmission dynamics

8.1. Background

The purpose of this chapter is to elucidate the effect livestock vaccination has on RVFV transmission dynamics. To do this a stochastic compartmental model was implemented. The role of a model is to make predictions or provide further understanding of disease transmission dynamics (Keeling and Rohani 2008). Models can provide predictions or understanding on disease occurrence, surveillance or impact of control strategies (Métras et al. 2011).

Models focusing on different fields of RVF epidemiology have been published including risk assessments, spatial epidemiology, predicting RVF emergence and transmission dynamics, although they vary considerably in complexity and scale (Métras et al. 2011). In particular a number of compartmental models assessing RVFV transmission dynamics have been published.

Most published models are deterministic (Danzetta et al. 2016). These models do not account for randomness, which is particularly important when an outbreak first occurs, when the number of infectious animals is small, when there is a trough phase of an epidemic or when a control measure has been implemented successfully (Keeling and Rohani 2008). Stochastic models work by running multiple simulations accounting for ‘chance’. However, relatively few stochastic models have been published for RVFV (Cavalerie et al. 2015, Sumaye et al. 2019, Métras et al. 2017).

Vaccination is a suitable control measure for RVF, and its impact can be assessed by using modelling (Gaff et al. 2011, Adongo et al. 2013, Chamchod et al. 2015, Gachohi et al. 2016). This chapter brings components of the previous chapters together to assess the impact livestock vaccination has on potential RVF outbreaks, livestock population dynamics and

maintenance of the virus over interepidemic years. In the absence of conducting a vaccine efficacy field trial which assesses protection against infection rather than immunogenicity, a transmission dynamics model is a way of exploring questions related to transmission, and the impact of strategies such as vaccination at a herd level and what effect this has on controlling viral incursions. The two vaccines evaluated in Chapter 7 (ChAdOx1 RVF and Smithburn) can be directly compared to assess their impact on RVFV transmission dynamics using the seroconversion results.

As reported in Chapter 3, the Machakos county has previously been reported as a high-risk area for RVFV transmission, although results from chapter 3 also show there is no RVF circulating on the ILRI Kapiti ranch. With this in mind, population sizes and rainfall data incorporated into the model are based on the Kapiti farm. An aim for this chapter is therefore to predict what would happen with regard to infections in herds on the farm, how vaccination could be implemented in an effective way and how this depends on the frequency of revaccination, which species are vaccinated and the efficacy of the vaccine.

8.2. Methods

8.2.1. Overview

To understand the basis of RVFV transmission dynamics it is fundamental to include the dynamics of infection in the primary vector species and ruminant host species (sheep and cattle) as well as climatic data. Ruminant host species are modelled separately: sheep are highly susceptible to the disease whilst cattle can often harbour the infection sub-clinically (Georges et al. 2018).

The model is adapted from a stochastic compartmental model for bluetongue virus transmission dynamics including two ruminant host species and one vector (Gubbins et al.

2008, Szmaragd et al. 2009, Sumner et al. 2017). The two host modules are divided into susceptible, infected (and infectious), recovered (S-I-R model) as well as vaccinated compartments. The vector module is divided into three compartments: susceptible, latent and infectious (S-L-I model) and there is also a vector egg module split into susceptible and infected compartments (S-I model). Movement between compartments are stochastic processes. To keep the model simple mosquito feeding on humans is not included. The biannual rainy seasons in Kenya are modelled as a proxy to guide vector activity and hence simulate recurring outbreaks used to assess the impact of RVF vaccination.

8.2.2. Forces of infection

The force of infection for host species X (λ_x) is a function of the probability of transmission from vector to host (b_x , where X is either C , cattle or S , Sheep), the vector feeding rate (a_x), the seasonal vector activity (θ), the vector-to-host ratio (m_x) and the proportion of infectious vectors (I), so that

Equation 8.1

$$\lambda_x = b_x \times a_x \times \theta(t) \times m_x \times \frac{I}{N}$$

The force of infection for vectors (λ_V) is a function of the probability of transmission from host to vector (beta_c , cattle or beta_s , sheep), the vector feeding rate (a_c or a_s), the seasonal vector activity (θ) and the ~~proportion~~ number of cattle (Y_j^C) and sheep (Y_j^S) that are in the infected stage, so that n_c

Equation 8.2

$$\lambda_V = \text{beta}_c \times a_c \times \theta \times \frac{1}{HC} \sum_{j=1}^{n_c} Y_j^C + \text{beta}_s \times a_s \times \theta \times \frac{1}{HS} \sum_{j=1}^{n_s} Y_j^S$$

8.2.3. Transitions

Transitions between compartments are stochastic processes (Table 8.1). The number of transitions of each type during a small-time interval (δt) was drawn from a binomial distribution with population size (n) and transition probability (q) (the appropriate per capita rate multiplied by δt).

Table 8.1 Transitions, probabilities and population sizes in the RVF transmission dynamics model. Where X^c is susceptible cattle, Y_j^c is infected cattle and Z^c is recovered cattle. The process is exactly the same for sheep (X^s, Y_j^s, Z^s). Y_j^c and L_j refer to the stage of viraemia the host/vector are in respectively, there are 3 stages of viraemia for both the host and vector.

Description	Transition	Probability	Population size
		Hosts [†]	
Infection	$\begin{cases} X^c \rightarrow X^c - 1 \\ Y_1^c \rightarrow Y_1^c + 1 \end{cases}$	$\lambda_c \delta t$	X_c
Completion of infection stage	$\begin{cases} Y_j^c \rightarrow Y_j^c - 1 \\ Y_{j+1}^c \rightarrow Y_{j+1}^c + 1 \end{cases}$	$n_c r_c \delta t$	Y_j^c
Mortality during infection stage	$\begin{cases} Y_j^c \rightarrow Y_j^c - 1 \\ X^c \rightarrow X^c + 1 \end{cases}$	$(bm_c + d_c) \delta t$	Y_3^c
Recovery	$\begin{cases} Y_3^c \rightarrow Y_3^c - 1 \\ Z^c \rightarrow Z^c + 1 \end{cases}$	$n_c r_c \delta t$	$Y_{n_c}^c$
Vectors			
Infection	$\begin{cases} S \rightarrow S - 1 \\ L_1 \rightarrow L_1 + 1 \end{cases}$	$\lambda_v \delta t$	S
Completion of extrinsic incubation period	$\begin{cases} L_j \rightarrow L_j - 1 \\ L_{j+1} \rightarrow L_{j+1} + 1 \end{cases}$	$k_v \delta t$	L_j
Vector mortality during EIP	$\begin{cases} L_j \rightarrow L_j - 1 \\ S \rightarrow S + 1 \end{cases}$	$\mu \delta t$	L_j
Completion of EIP	$\begin{cases} L_k \rightarrow L_k - 1 \\ I \rightarrow I + 1 \end{cases}$	$k_v \delta t$	L_k
Mortality of infectious vectors	$\begin{cases} I \rightarrow I - 1 \\ S \rightarrow S + 1 \end{cases}$	$\mu \delta t$	I

[†] host transitions are shown for cattle; those for sheep are the same, but with S replacing C
Adapted from Sumner et al. 2017

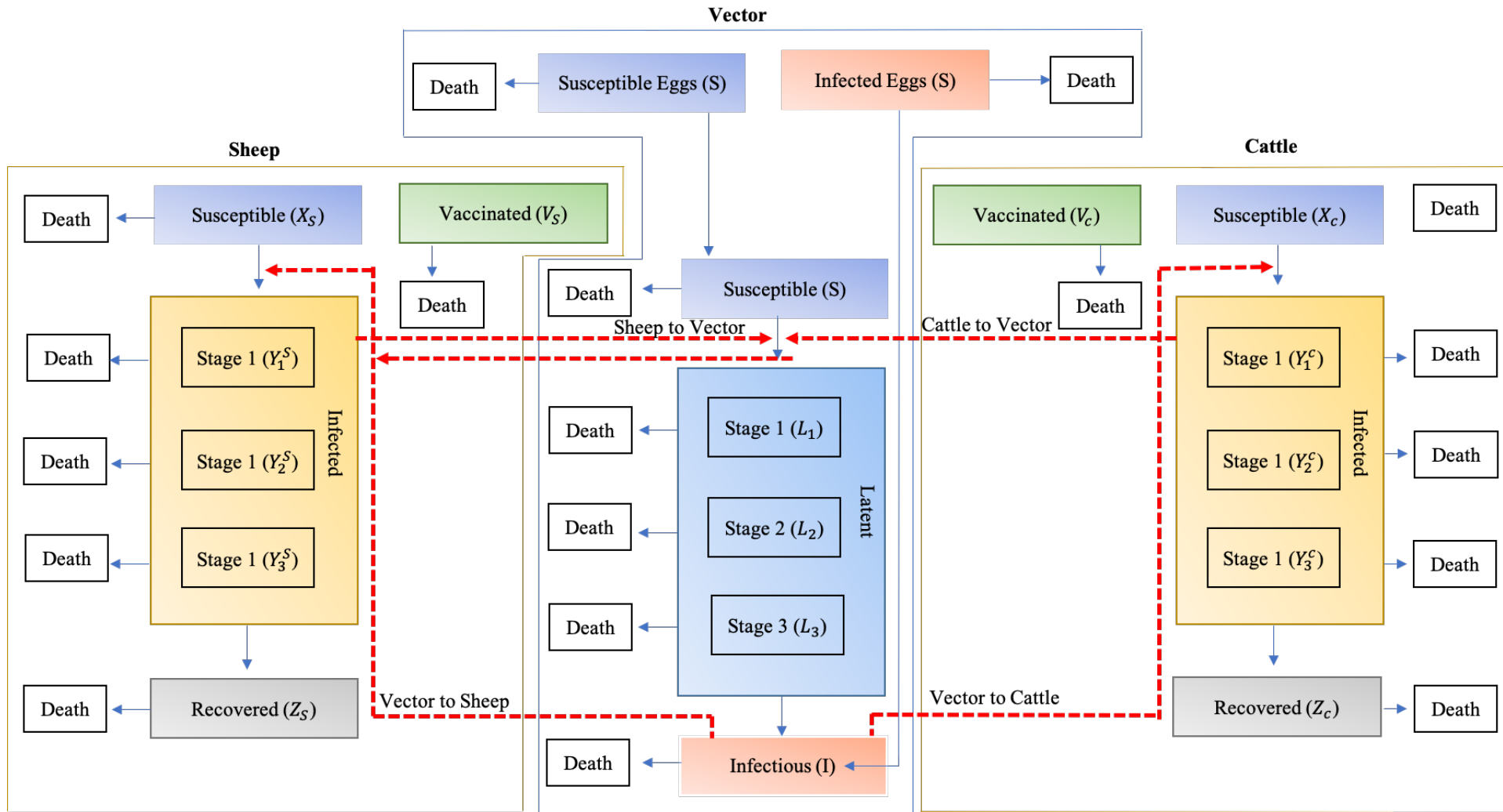


Figure 8.1 Model structure demonstrating RVF transmission pathways and infection states for two livestock species and one mosquito vector.

8.2.4. Assumptions

1. A time step denotes one day in the year, for example if $t_{\text{Start}}=120$, the start of the simulation was on 30th April.
2. Vaccine efficacy (p_{VX}) is based on vaccine immunogenicity results from the vaccine field trial in Chapter 7. Using the vaccine immunogenicity results from both the ChAdOx1 RVF vaccine and the Smithburn vaccine.
3. The total numbers of cattle (HC) and sheep (HS) are based on animal numbers of the field trial study farm. The ratio between the different animal species is similar to what others have used for RVF transmission dynamics models based in Kenya (Gachohi et al. 2016).
4. Both livestock populations are assumed to be constant with births assumed to match deaths (mortality due to RVF and other causes).
5. To account for transovarial transmission, number of eggs laid per day (n_{EggsD}) was parameterised but again was kept constant along with vector mortality rates.
6. Vector activity was based on traditional understanding of biannual rainfall patterns in Kenya. Vector activity (egg hatching) only occurs during the rainy seasons. Outside of the rainy season, there is no vector activity. Furthermore, the model also accounts for rainfall intensity; if annual rainfall exceeds a certain threshold the intensity of vector activity (egg hatching, force of infection for vectors) will increase. The model does not include any effect of temperature on vector activity.
7. The model accounts for primary vector transmission (*Aedes* spp.) only. The primary vector is important for RVF transmission dynamics as research suggests it is able to transmit the virus transovarially into eggs (Linthicum et al. 1985).

A table of all model parameters and their suitability can be found in Table 8.2. Suitability was based on the primary research and how relevant it was to the main aim of the

parameter. The suitability score was based on a range from 0-3 with 0=Poor and 3=Good. An example is the probability of transmission from vector to cattle, the value of this parameter came from a paper that analysed transmission from *Aedes* and *Culex* mosquitoes to hamsters. This value was scored 2 as the experiment was done on a mammalian species but not the target species for the model (cattle or sheep). The suitability column provides rationale for the parameters used in the model and where research should be targeted further.

Table 8.2 RVF Transmission Model Parameters and their suitability

Description	Symbol	Value	Suitability *
Cattle Parameters			
Total cattle population	HC	2907	3
Probability of transmission cattle to vector	β_{c_v}	0.19 (daily)	2
Reciprocal of mean duration of viraemia Cattle	r_c	1/6 (days)	
Number of stages of viraemia	n_c	3	2
Vaccine efficacy for cattle	pV_c	0, 30, 50, 90 (%)	-
- ChAdOx1 RVF		76.3 (%)	3
- Smithburn		3.8 (%)	3
RVFV induced mortality adult cattle	d_c	0.075 (daily)	3
Baseline mortality cattle	bm_c	0.000611 (daily)	3
Sheep Parameters			
Total sheep population	HS	1415	3
Transmission sheep to vector	β_{s_v}	0.22 (daily)	2
RVFV induced mortality adult sheep	d_s	0.2 (daily)	3
Reciprocal of mean duration of viraemia Sheep	r_s	1/3 (days)	
Number of stages of viraemia sheep	n_s	3	2
Vaccine efficacy for sheep	pV_s	0, 30, 50, 90 (%)	-

- ChAdOx1 RVF		94.9 (%)	3
- Smithburn		16.3 (%)	3
Baseline mortality sheep	bm_s	0.000814 (daily)	3
Vector Parameters			
Mosquitoes per host	m	24	1
Number of adult mosquitoes	N	$m^*(HC+HS)$	3
Probability of transmission from vector to cattle	b_c	0.14	2
Mosquito to cattle ratio	m_c	N/HC	2
Vector preference for cattle	ϕC	0.75	2
Biting rate	a	0.33	1
Vector feeding rate for cattle	a_c	$a*\phi C$	2
Probability of transmission from vector to sheep	b_s	0.14	2
Mosquito: sheep ratio	m_s	N/HS	2
Vector preference for sheep	ϕS	0.25	2
Vector feeding rate sheep	a_s	$a*\phi S$	2
Number of stages of EIP	k	3	2
Reciprocal of the mean EIP	v	1/3	3
Mortality rate of vector	μ	0.1 (daily)	3
Probability of transovarial transmission	$prTOT$	0.1	1
Daily survival probability for eggs	$prSurv$	0.996 (/day)	2
Number of eggs laid per day	$nEggD$	62.5 (/day)	3

*Suitability ranges from 0-3 with 0=Poor and 3=Good

8.2.5. Host parameters

Probability of transmission from host to vector (β_{x_i})

The probability of transmission from cattle to vector (β_c) was different from the probability of transmission from sheep to vector (β_s). The transmission from host to vector (host competence) was calculated in a previous study that calculated a vertebrate host competence index accounting for vertebrate viraemia (virus titre and duration of viraemia) and mosquito susceptibility to the virus (Golnar et al 2014). The competence represents the relative number of infectious mosquitoes that may result from feeding on infected vertebrate hosts. Quantified viral titre data was sourced from the published literature.

Due to limited species-specific transmission data the equation was based on pooled transmission data of 17 mosquito species. The daily infectiousness of the vertebrate hosts was calculated by including the daily vertebrate host viraemia titres with the infectious mosquitos feeding in the model. Those results that were negative were set to zero. Using the host's viraemic period the daily values from the equation were summed and a vertebrate species competence index was calculated. Values were calculated for specific livestock species: for probability of transmission from cattle to vector $\beta_c = 0.19$ and for the probability of transmission from sheep to vector $\beta_s = 0.22$ (Golnar et al. 2014).

Reciprocal of mean duration of viraemia (r_x)

The infectious period typically ranges from 3 to 6 days (Bird et al. 2009, Pepin et al. 2010). Gachohi et al. (2016) used 6 days for infectious period for both hosts; this value was used for cattle. A study in sheep showed the peak value for viraemia occurred 3 days after infection and this value was used for sheep (Faburay et al. 2016). Therefore the reciprocal of mean duration of viraemia for cattle (r_c) is 1/6 and for sheep (r_s) it is 1/3.

Number of stages for viraemia (n_x)

In epidemiological models infectious periods have traditionally been described by exponential distributions. However, this assumes that the time to recovery is independent of the time since infection. In this model the duration of viraemia is split into three stages to provide a general gamma distribution. Newly infected hosts enter the first stage and then pass through each further stage successively. By doing this it provides a more realistic distribution of the infectious period and recovery process in the hosts (Lloyd 2001, Gubbins et al. 2008). The total length of time in each stage follows a gamma distribution.

Vaccine efficacy (pV_c)

Vaccine efficacy was estimated from vaccine immunogenicity results from Chapter 7 (pV_c). In addition, different values were used to assess the impact of vaccine efficacy on the transmission dynamics. This parameter was separate for cattle and sheep to account for species-specific differences in vaccine effectiveness.

Disease-associated mortality rate for the host (d_x)

The RVFV-associated mortality rate for sheep and cattle differed. This is because sheep are more susceptible than cattle. For cattle the mortality rate was 0.075 based on evidence that the mortality rate is between 5-10% for adult cattle (Bird 2009, Gachohi et al. 2016). The RVFV mortality rate for sheep was estimated as 0.2. Bird et al. (2009) described the mortality ratios in adult sheep as 10-30% and Easterday et al. (1962) found that 20% of adult sheep died following experimental RVFV infection. Both of these values for RVF induced mortality have been used in previous mathematical models (Chamchod et al. 2015, Gachohi et al. 2016).

Baseline mortality rate (bm_x)

The baseline mortality rate is the average mortality rate of cattle or sheep that die of causes other than RVF. Mortality rates vary for different types of cattle (dairy, beef, etc.) and different production systems (small scale, large scale), but on average cattle live for 3-6 years in Kenya (Mwanyumba et al. 2015). Age of mortality can be a lot higher, but for selling on, cattle are normally slaughtered around 3 years old (Alarcon et al. 2017). In the model a mortality rate of 0.000611 is used for cattle, this equates to a mean lifetime of 4.5 years and has been used in transmission dynamics models previously (Gachohi et al. 2016). In the model a mortality rate of 0.000814 is used for sheep, which is a mean lifetime of 3.3 years (Gachohi et al. 2016).

8.2.6. Vector parameters

Probability of transmission from vector to host (b_x)

The probability of RVFV transmission from vector to cattle (b_c) and sheep (b_s) was based on disseminated RVFV infection in *Aedes aegypti* and *Aedes mcintoshi* mosquito transmission into hamsters (Turell et al. 2008). Although not based on the target host species (sheep or cattle), it does provide primary data on transmission from mosquitoes to other animals. This value comes from a study that addressed vector competence in different African mosquitoes: the parameter provided is based entirely on transmission potential, other parameters accounting for biting rate and vector preference. This value has been used previously in other transmission dynamics models (Chamchod et al. 2015, Gachohi et al. 2016).

Vector-to-host ratio (m_x)

The mosquito to host ratio (m_x) was derived from the total number of mosquitoes (N) divided by the total host population (cattle HC and sheep HS separately). The total number of mosquitoes (N) is calculated by an assumed mosquitoes per host ($m=24$) times the sum of

total cattle and total sheep. The assumed mosquitoes per host (m) was estimated from *Aedes*:Host ratio graphs from Gachohi et al. (2016).

Vector feeding rate (a_x)

The vector biting rate (a) is estimated from a number of studies. A mathematical model has been constructed for Zika virus to assess the effect of *Aedes aegypti* and *Aedes albopictus* biting rates on transmission dynamics. The model estimated that daily biting rates were between 0.01 and 0.35 (Suparit et al. 2018). In another paper the biting rate for *Culex* spp. in relation to West Nile Virus transmission was estimated as 0.32 (Durand et al. 2010). Gachohi et al. (2016) used a value of 0.33, which has also been used in this present model. Three times as many mosquitoes bite cattle than sheep (Tchouassi et al. 2016), this was accounted for in the vector feeding rate parameter ϕX .

Seasonal vector activity (θ)

There is an intrinsic relationship between rainfall, vector activity and viral emergence as reported for many vector-borne diseases. Rainfall has a direct impact on RVFV transmission dynamics as *Aedes* mosquitoes typically emerge following heavy persistent rainfall and flooding (Linthicum et al. 1985, Sang et al. 2017). The seasonal vector activity (θ) parameter is described as a binary variable that is only activated during the rainy seasons, so that

Equation 8.3

$$\theta(t) = \begin{cases} 0 & t \in \text{dry season} \\ \max\theta & t \in \text{rainy season} \end{cases}$$

The intensity of the vector activity ($max\theta$) is dependent on the annual rainfall level for that year. If the annual rainfall is above the threshold level ($rainThresh$) the vector activity will be high and if it is below the threshold level vector activity it would be low. The threshold level was set based on the average rainfall (50cm) that falls in Kapiti.

Rainfall data

Annual rainfall data was incorporated into the model to drive vector activity and, hence, the force of infection between the mosquitoes and livestock hosts. Kenya typically has a biannual rainfall pattern; rain falls between April and May and November and December. Vector activity occurred in two parts: if the time was set during the rainy season (day 50 to day 151 or day 250 to day 365), vector activity would commence and if the annual amount of rainfall reached a defined threshold ($rainThresh$) the intensity of vector activity would increase. The rationale for this lies in the notion that RVFV outbreaks occur when there are heavier than normal amounts of rainfall (Davies et al. 1975). Existing rainfall data was sought from the Kapiti farm, with this data the model could run for 9125 days (25 years) which included multiple known outbreak years in the country.

Extrinsic Incubation Period (EIP) ($1/\nu$)

The extrinsic incubation period is the time taken for the virus to develop within a vector: infect host cells, replicate and disseminate (Lumley et al. 2017). It has previously been estimated that 3 days are needed for the extrinsic incubation period for RVFV in *Aedes* and *Culex* species of mosquitoes (Turell 1985).

Number of stages for EIP (k)

Similar to viraemia in the host, the EIP is separated into stages (k) to allow for a more general gamma distribution. Infected mosquitoes pass through each stage successively.

Vector mortality rate (μ)

The mortality rate of vectors (μ) is 0.1, this is the reciprocal of the lifespan. This value was based on the life expectancy of *Aedes aegypti* females being 9.2 days (Muir and Kay 1998).

Number of eggs laid per day (nEggD)

Aedes mosquitoes lay approximately 100-150 eggs in every batch (Clemons et al. 2010) and they tend to have 5 batches in their lifetime. This means they lay approximately 500-750 eggs in their lifetime. To calculate number of eggs laid per day the mean lifetime eggs laid was divided by the mean lifespan of the mosquitoes (62.5 eggs per day). This is supported by a paper that assessed parity rates in *Aedes aegypti* mosquitoes daily (Goindin et al. 2015). The model does not include dynamics for eggs that persist in the environment for multiple years before hatching.

Probability of eggs surviving (prSurv)

The parameter for daily probability of eggs surviving (hatching) is based on eggs that survive to the next season to hatch. This parameter is based on research showing that *Aedes* mosquito eggs laid within the last 4 weeks of the rainy season are able to overwinter and about 0.67 (2/3) of these will survive to hatch at the next rainy season (Favier et al. 2006, Chitnis et al. 2014). Assuming a dry season of 100 days, this gives a daily survival probability of $0.67^{1/100}=0.996$.

Probability of transovarial transmission from infected female to egg (prTOT)

The principal theory of long-term maintenance of RVFV in the environment involves transovarial transmission of the virus from infected females into their eggs. Research has shown that unengorged mosquitoes were found to be positive to RVFV in Kenya (Linthicum et al. 1985), which provides evidence that RVFV can be transmitted transovarially. Further

research into virus-infected mosquito eggs supports the link with endemic maintenance (Romoser et al. 2011). An assumed value of 0.1 was used as the proportion of transovarial transmission from infected female to egg (*prTOT*). There is little in the literature about the proportion of eggs that become infected with RVFV through transovarial means. This value has been used in a model specifically looking at transovarial transmission of RVFV (Chitnis et al. 2013).

8.2.7. Different vaccination scenarios tested

Firstly, the model was run without any vaccination as a baseline for comparison. Four overarching vaccination scenarios were assessed:

1. Seasonality of vaccination: In one scenario annual vaccination was conducted in the rainy season (in response to an outbreak) in another scenario vaccination was conducted in the dry season (pre-emptive vaccination). Vaccination was conducted annually over 20 years. The scenarios assessed what impact three different theoretical vaccine efficacies: 30%, 50%, 90% had on the reduction of RVF cases in cattle and sheep.
2. Species-specific vaccination: the same theoretical vaccine efficacies used to assess effect of seasonality were also used to assess the impact of species-specific vaccination e.g. does vaccinating cattle only have any impact on reduction of RVF cases in sheep (vice-versa)? This was on annual vaccination during the dry season over the 20 years.
3. Impact of ChAdOx1 RVF and Smithburn vaccination: In this scenario vaccine immunogenicity results for ChAdOx1 RVF and Smithburn vaccine from Chapter 7 were used to assess the impact on reduction of number of RVF cases in cattle and sheep when vaccinated annually during the dry season over a 20 years period.

4. Frequency of revaccination with the ChAdOx1 RVF vaccine: This scenario tested re-vaccination strategies for every 3, 5 and 8 years to see what impact this has on reduction of cattle and livestock RVF cases. Vaccination was undertaken in the dry season over the 20-year period.

Table 8.3 Vaccination Scenarios used to assess reduction of cumulative incidence of RVF cases in cattle and sheep

Model Scenarios		Figure
Baseline		
No vaccination		8.3
1. Seasonal vaccination		
Theoretical annual vaccination efficacies (%) in rainy season over a 20- year period	30%	8.5
	50%	8.5
	90%	8.5
Theoretical annual vaccination efficacies (%) in dry season over a 20- year period	30%	8.6
	50%	8.6
	90%	8.6
2. Species-specific vaccination		
Cattle only and Sheep only theoretical vaccination efficacies (%) in dry season over a 20-year period	30%	8.7
	50%	8.7
	90%	8.7
3. Field Trial vaccine efficacy		
Vaccination occurred annually during dry season over a 20-year period		
ChAdOx1 RVF vaccine efficacy in Cattle (%)	76.3%	8.8
ChAdOx1 RVF vaccine efficacy in Sheep (%)	94.9%	8.8
Smithburn vaccine efficacy in Cattle (%)	3.8%	8.8
Smithburn vaccine efficacy in Sheep (%)	16.3%	8.8
4. Re-vaccination		
Frequency of re-vaccination (years) with ChAdOx1 RVF vaccine during dry season over a 20-year period	3 years	8.11
	5 years	8.11
	8 years	8.11

8.2.8. Statistical analysis of model outputs

Kruskall-Wallis tests were used to see if there was any significant difference in model predictions for outbreak size (cumulative incidence of infected hosts) for different vaccine efficacies or revaccination intervals. All model development and analysis were conducted on R (version 3.3.3) (R Core team 2013). Table 8.3 outlines the outputs that were assessed from the model.

8.3. Results

8.3.1. Rift Valley fever virus transmission dynamics

Initially, the model was run without vaccination to show the transmission dynamics of RVF in sheep and cattle without control. RVF was introduced via infected mosquitoes on day 121, which is during the rainy season. During that year rainfall was higher than the threshold and an outbreak occurred (Figure 8.2a and b). Figure 8.2 shows the median number of infected sheep (Figure 8.2c) and cattle (Figure 8.2d) over the 24-year period from the 100 replicates along with the total rainfall for each year modelled. Outbreaks of infected cattle and sheep were associated with heavy rainfall, although large outbreaks did not occur every time rainfall was above the threshold, often because there were few susceptible animals (Figure 8.2 e and f). The first outbreak occurred in the first year modelled causing 74% of cattle and 50% sheep to become infected. Large outbreaks occurred just before year 3, 8, 12 and year 16. Transient outbreaks occurred in subsequent years after major outbreaks. The peak in infected cattle and sheep during these subsequent years was much less than the first year, ranging from 12.7%-23.8% in cattle and 9.6%-18.4% in sheep. The mean duration of outbreaks for cattle was 113 days for cattle and 97 days for sheep. The median cumulative incidence of infected cattle was 22,263 (Figure 8.3a) and for sheep was 14,707 (Figure 8.3b). The median cumulative incidence of RVF-related mortality in cattle was 7569 (Figure 8.4a) and in sheep 6202 (Figure 8.4b).

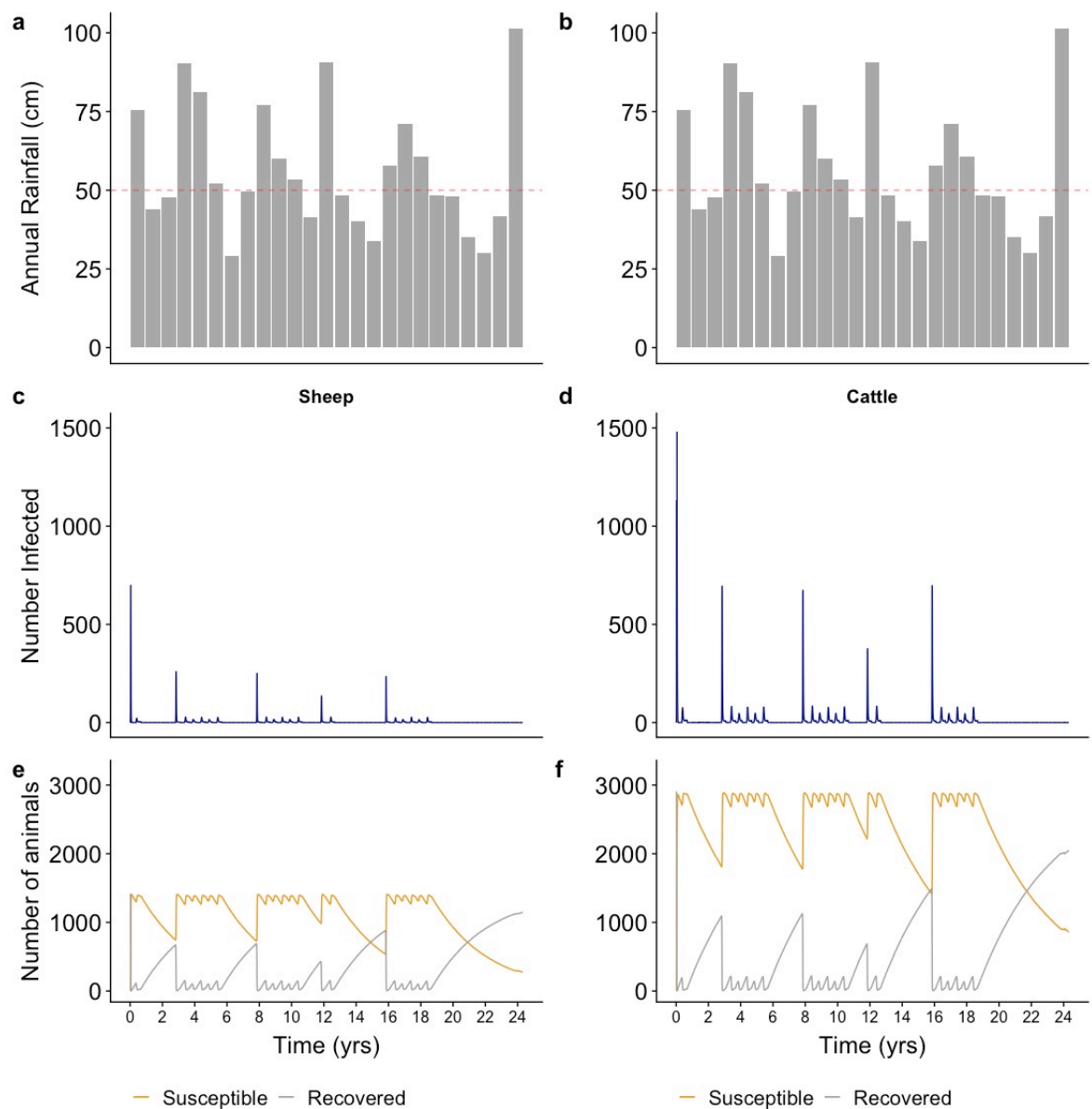


Figure 8.2 Transmission dynamics of Rift Valley fever virus in a population of sheep and cattle over a 24-year period. (a,b) Annual precipitation driving outbreaks. The red dashed line indicates the threshold for high levels of vector activity. (c,d) Number of infected (c) sheep and (d) cattle. (e,f) Number of susceptible (yellow) and recovered (grey) (e) sheep and (f) cattle. Results show the median of 100 replicates of the model.

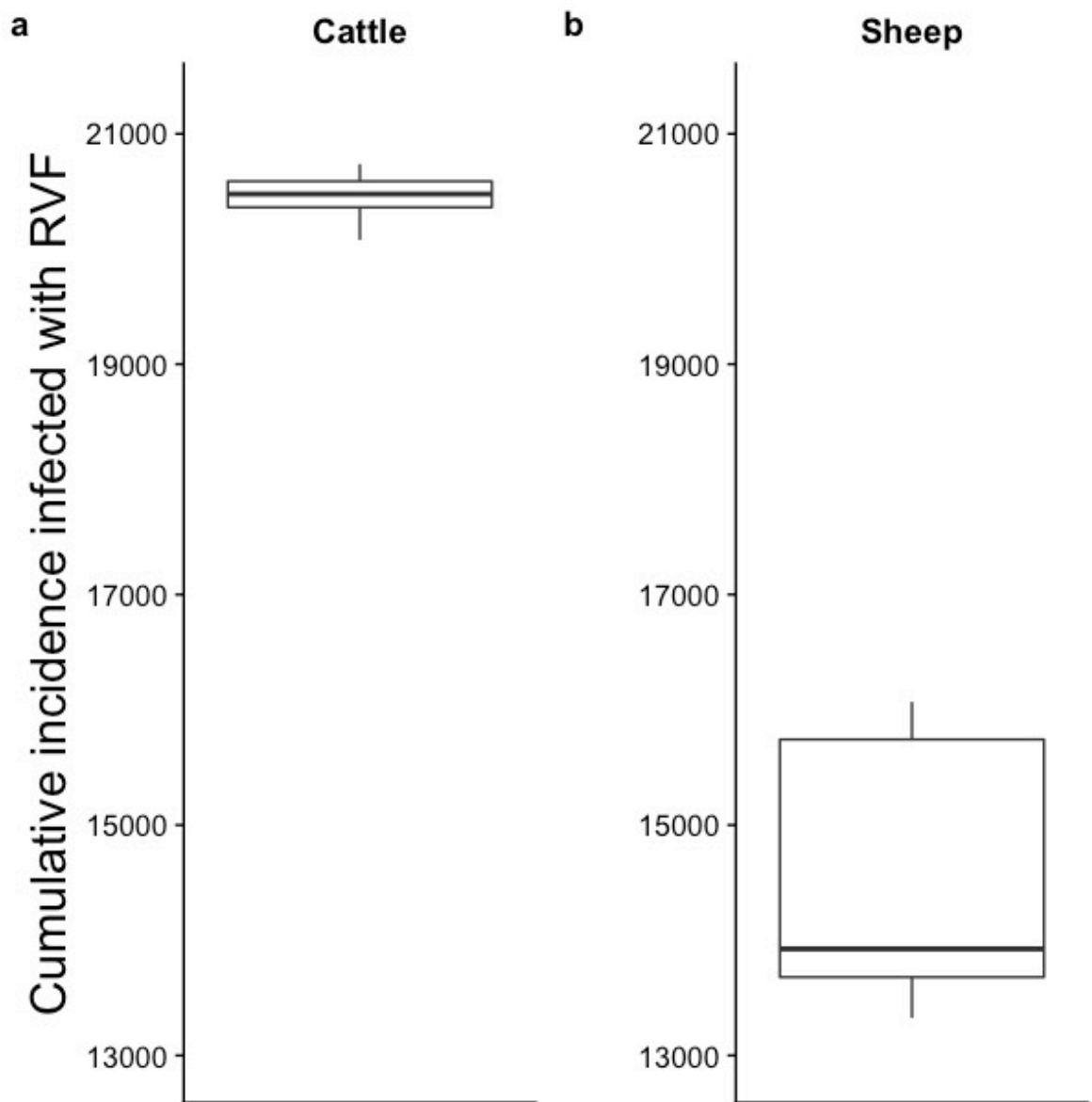


Figure 8.3 Cumulative incidence of RVF infected cattle (a) and sheep (b) during RVF transmission across a 24-year period. Box and whiskers show the median and interquartile range of 100 replicates of the model.

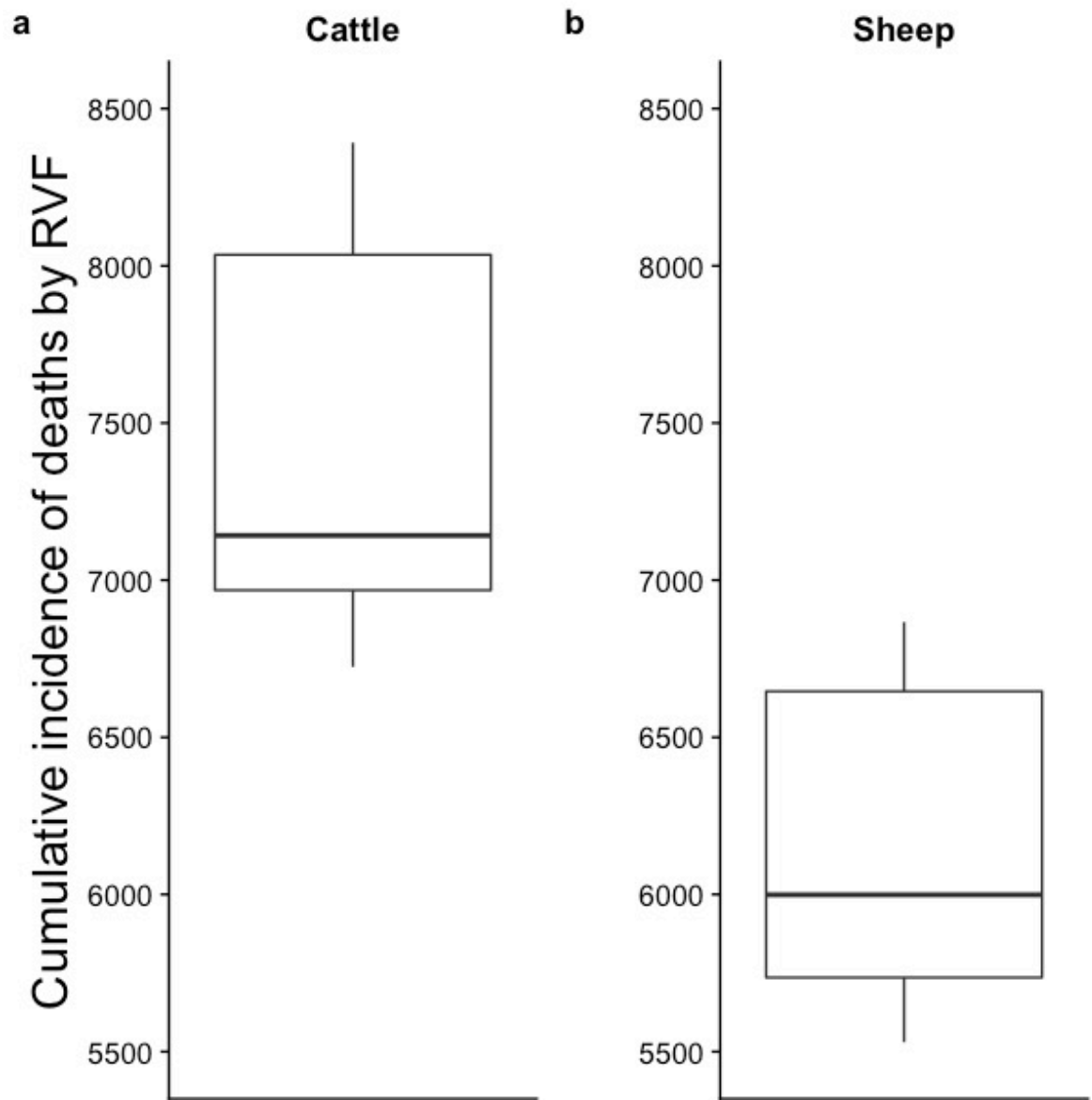


Figure 8.4 Cumulative incidence of deaths from RVF for cattle (a) and sheep (b) during RVF transmission across a 24-year period. Box and whiskers show the median and interquartile range of 100 replicates of the model.

8.3.2. Assessing vaccine efficacy during and prior to rainy seasons

Figure 8.5 shows the expected impact of annual vaccination on cumulative incidence of infected cattle (Figure 8.5a) and sheep (Figure 8.5b) during the rainy season (and hence in response to an outbreak) at different levels of vaccine coverage (no vaccine, 30%, 50% and 90%). The vaccination day was set as day 135 which falls within the first rainy season of the year and subsequently numerous outbreaks over the 24 years. The median number of infected cattle and sheep decreased with increasing vaccine efficacy (cattle Kruskal-Wallis: $\chi^2=99.27$, $p<0.001$; sheep, Kruskal-Wallis: $\chi^2=115.25$, $p<0.001$). However, there was substantial variability for all efficacies, and the range of cumulative infected cattle and sheep did overlap for the different vaccine efficacies as well as when no vaccination was given.

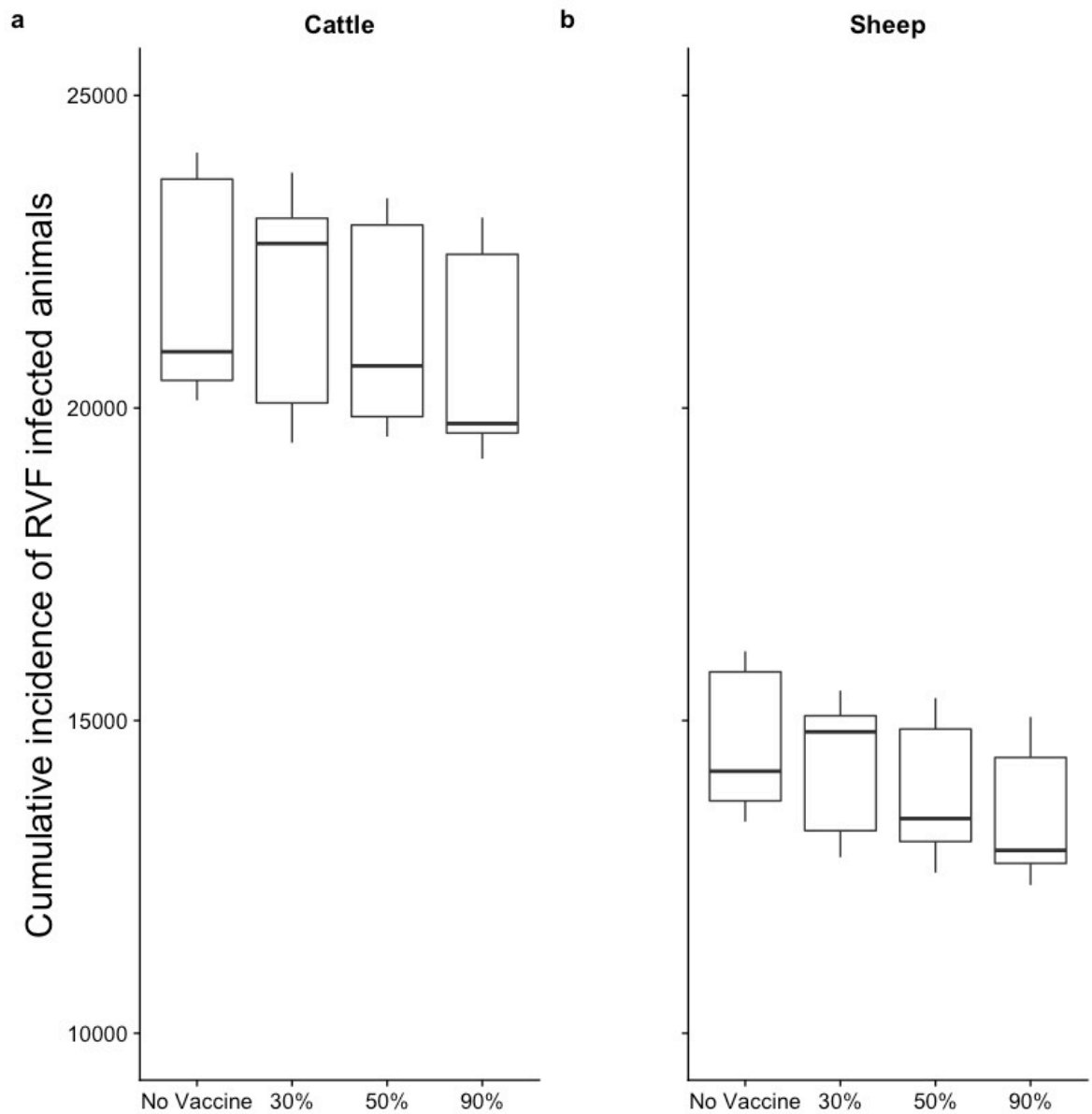


Figure 8.5 Cumulative incidence of RVF infected cattle (a) and sheep (b) across a 24-year period when animals are vaccinated during rainy season with different vaccine efficacies: 30%, 50% and 90% (Seasonal vaccination scenario, rainy season, Table 8.3). Box and whiskers show the median and interquartile range of 100 replicates of the model.

Figure 8.6 shows the expected impact of annual vaccination on cumulative incidence of infected cattle (Figure 8.6a) and sheep (Figure 8.6b) during the dry season at different levels of vaccine coverage (no vaccine, 30%, 50% and 90%). The vaccination day was set as day 200 which falls after the first rainy season of the year (and so after the initial outbreak). This could therefore be considered to be pre-emptive vaccination. The main finding is that the higher the vaccine efficacy the greater the reduction in cumulative incidence of cattle and sheep. There is a significant difference in numbers of cumulative infected cattle and sheep when vaccinating with different vaccine efficacies compared to no vaccination (cattle, Kruskal-Wallis $\chi^2=358.12$, $p<0.001$; sheep, Kruskal-Wallis: $\chi^2=255.78$, $p<0.001$). A vaccine efficacy of 90% reduces the median cumulative infected sheep by 5970 and 8900 cattle compared to not vaccinating at all.

8.3.3. Species specific vaccination

Vaccinating cattle only at different levels of vaccine efficacy had no effect on cumulative incidence of infected sheep (Kruskal-Wallis: $\chi^2=3.94$, $p=0.27$) (Figure 8.7a and b). Similarly, when vaccinating sheep only at different levels of vaccine efficacy, there was no effect on cumulative incidence of infected cattle (Kruskal-Wallis: $\chi^2=4.1823$, $p=0.24$) (Figure 8.7c and d). It is worth noting that vaccination did have an effect on the species that was vaccinated (Figure 8.7a and d).

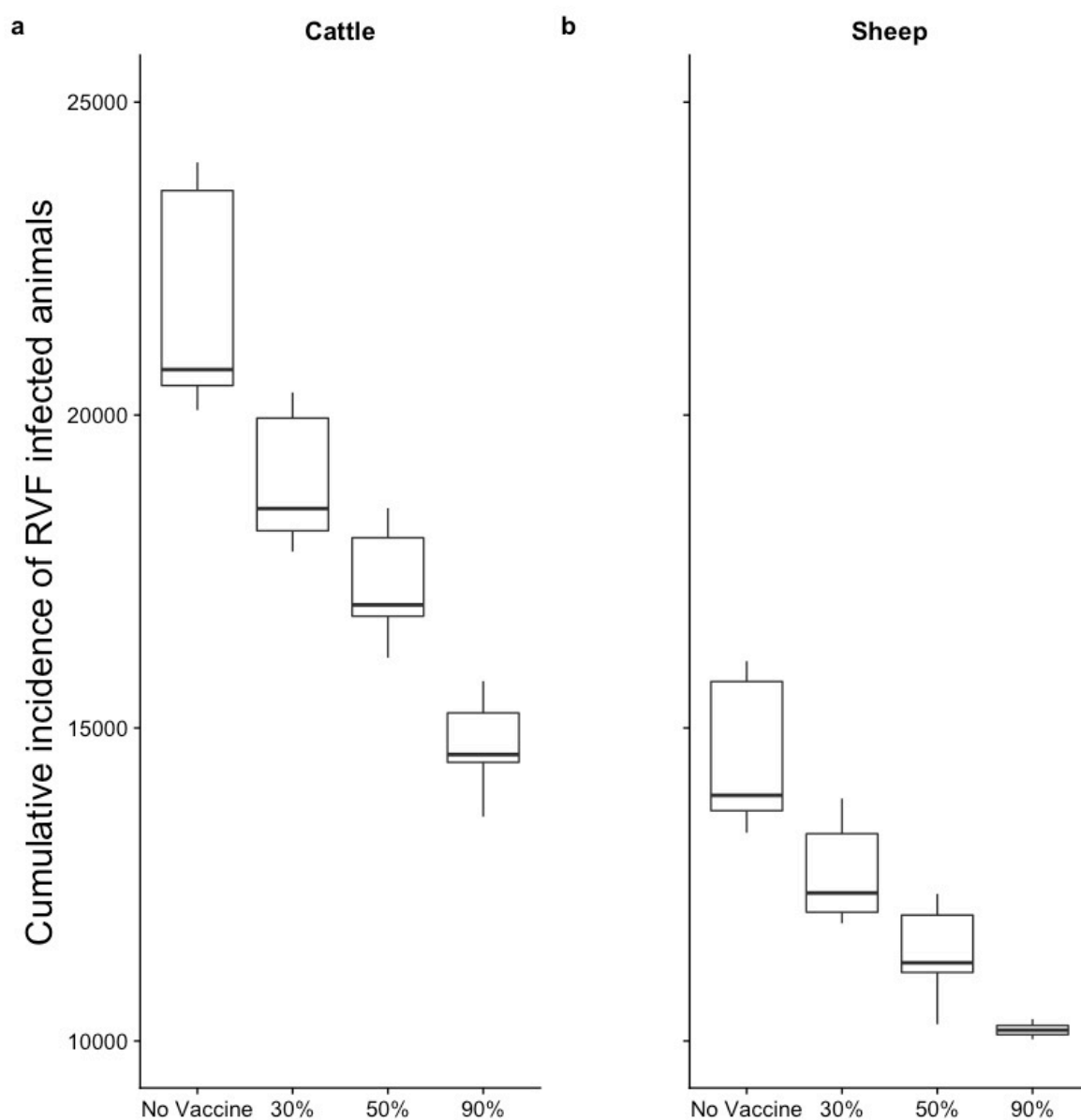


Figure 8.6 Cumulative incidence of RVF infected cattle (a) and sheep (b) across a 24-year period when animals are vaccinated during dry season with different vaccine efficacies: 30%, 50% and 90% (Seasonal vaccination scenario, dry season, Table 8.3). Box and whiskers show the median and interquartile range of 100 replicates of the model.

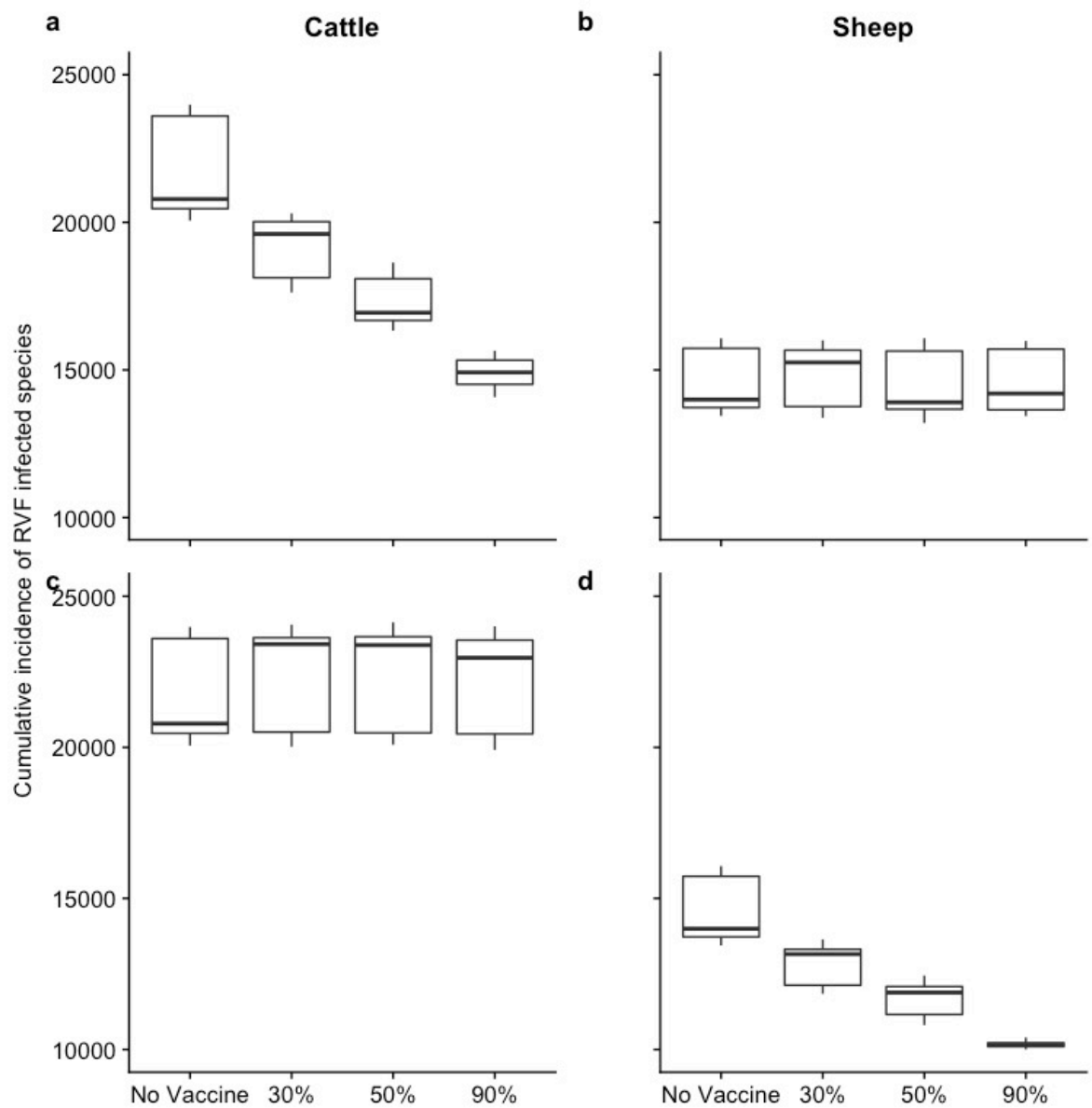


Figure 8.7 Effect of vaccine efficacy on cumulative incidence of RVF infected cattle and sheep when vaccinating cattle alone (a and b) and when vaccinating sheep alone (c and d) during the dry season. Vaccine efficacies analysed include 30%, 50%, 90% (Species-specific vaccination scenario, Table 8.3). Box and whiskers results show the median and interquartile range of 100 replicates of the model.

8.3.4. Impact of ChAdOx1 RVF and Smithburn vaccination on RVF transmission dynamics

Using the vaccine seroconversion results for the cattle and sheep vaccinated with ChAdOx1 RVF and Smithburn from Chapter 7, transmission dynamics were modelled to assess the impact of these vaccines. Vaccinations were conducted at day 200, in the dry season and re-vaccination occurred annually. Figure 8.8 shows the superior impact of ChAdOx1 RVF in reducing cumulative infected cattle and sheep (cattle, Kruskal-Wallis: $\chi^2=265.87$, $p<0.001$; sheep, Kruskal-Wallis: $\chi^2=265.38$, $p<0.001$). ChAdOx1 RVF vaccination prevented 5447 infections in cattle and 4574 infections in sheep. The Smithburn vaccine did not reduce cumulative incidence of infected cattle or sheep (Figure 8.8) and transmission dynamics over the 24-year period were similar to when there is no vaccine (Figure 8.9). When the ChAdOx1 RVF vaccine is used any outbreaks are minimal and where outbreaks do occur this is a product of new susceptible animals which have not been vaccinated (Figure 8.10). The duration of outbreaks did not change for either vaccine.

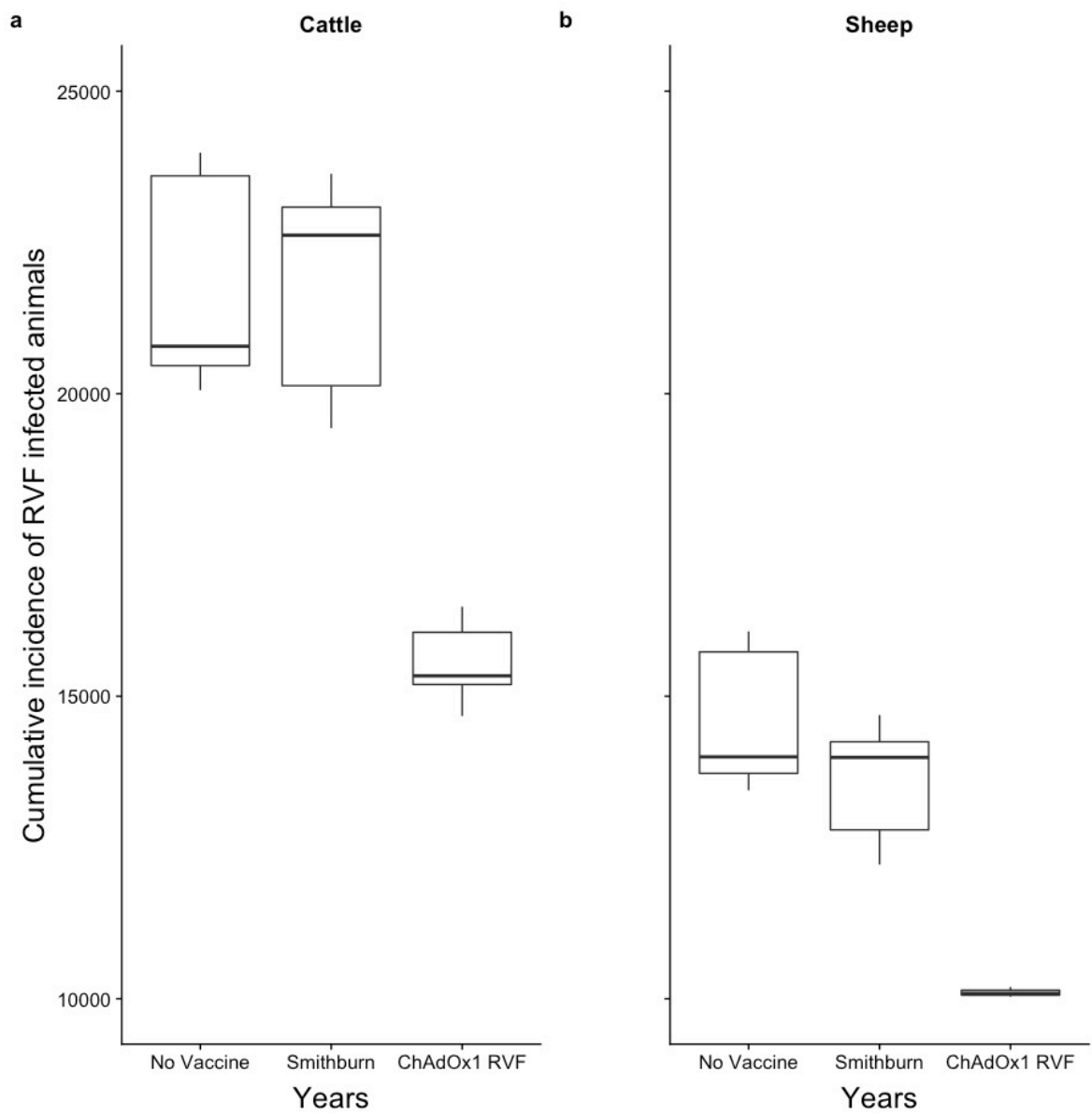


Figure 8.8 Effect of Smithburn or ChAdOx1 RVF vaccination on cumulative incidence of infected animals when vaccinating in dry season (Field trial vaccine efficacy scenario, Table 8.3). Box and whiskers show the median and interquartile range of 100 replicates of the model.

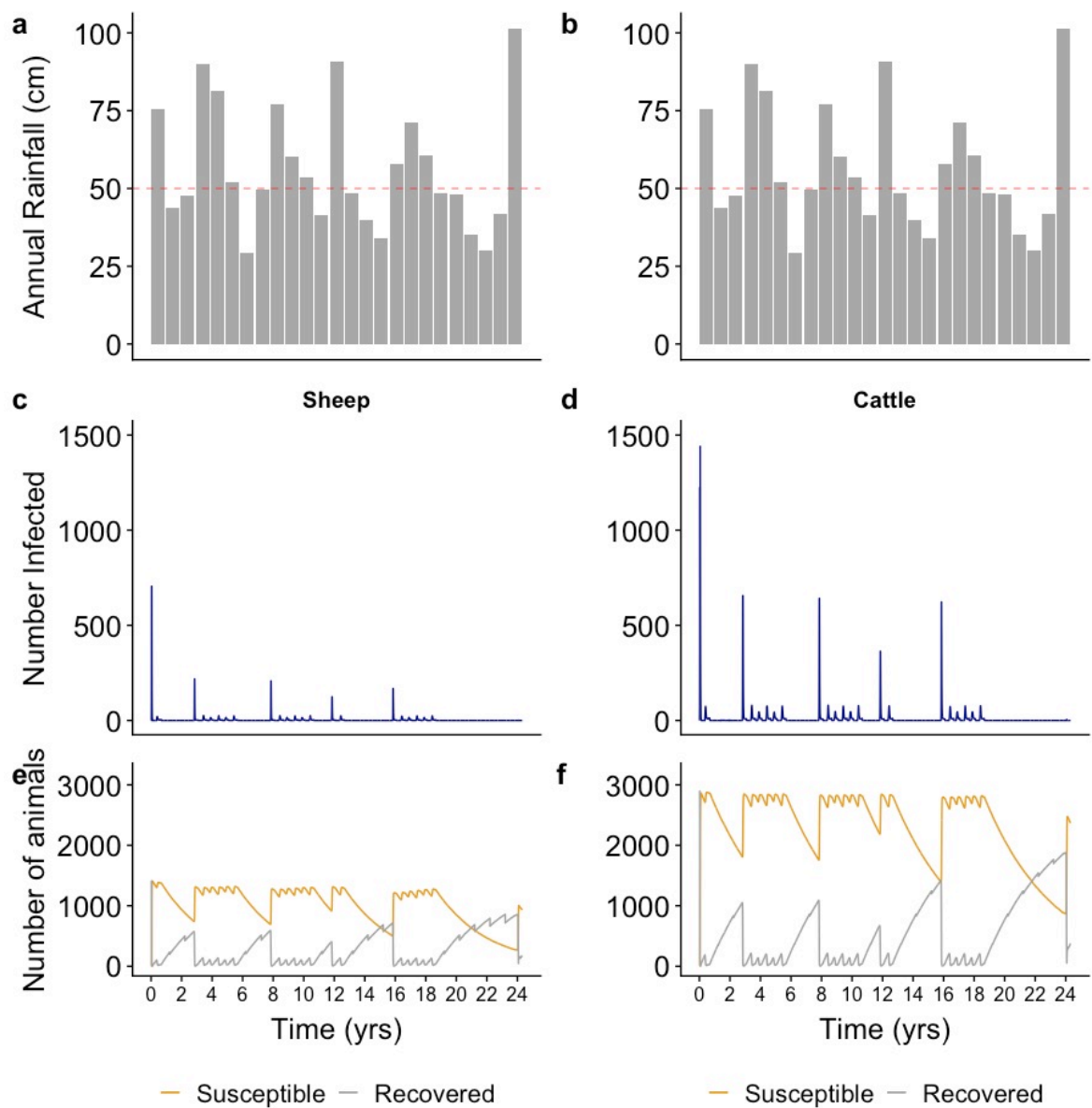


Figure 8.9 Transmission dynamics of Rift Valley fever virus in a population of sheep and cattle over a 24-year period when vaccinated with Smithburn vaccine. (a,b) Annual precipitation driving outbreaks. The red dashed line indicates the threshold for high levels of vector activity. (c,d) Number of infected (c) sheep and (d) cattle. (e,f) Number of susceptible (yellow) and recovered (grey) (e) sheep and (f) cattle. Results show the median of replicates of the model.

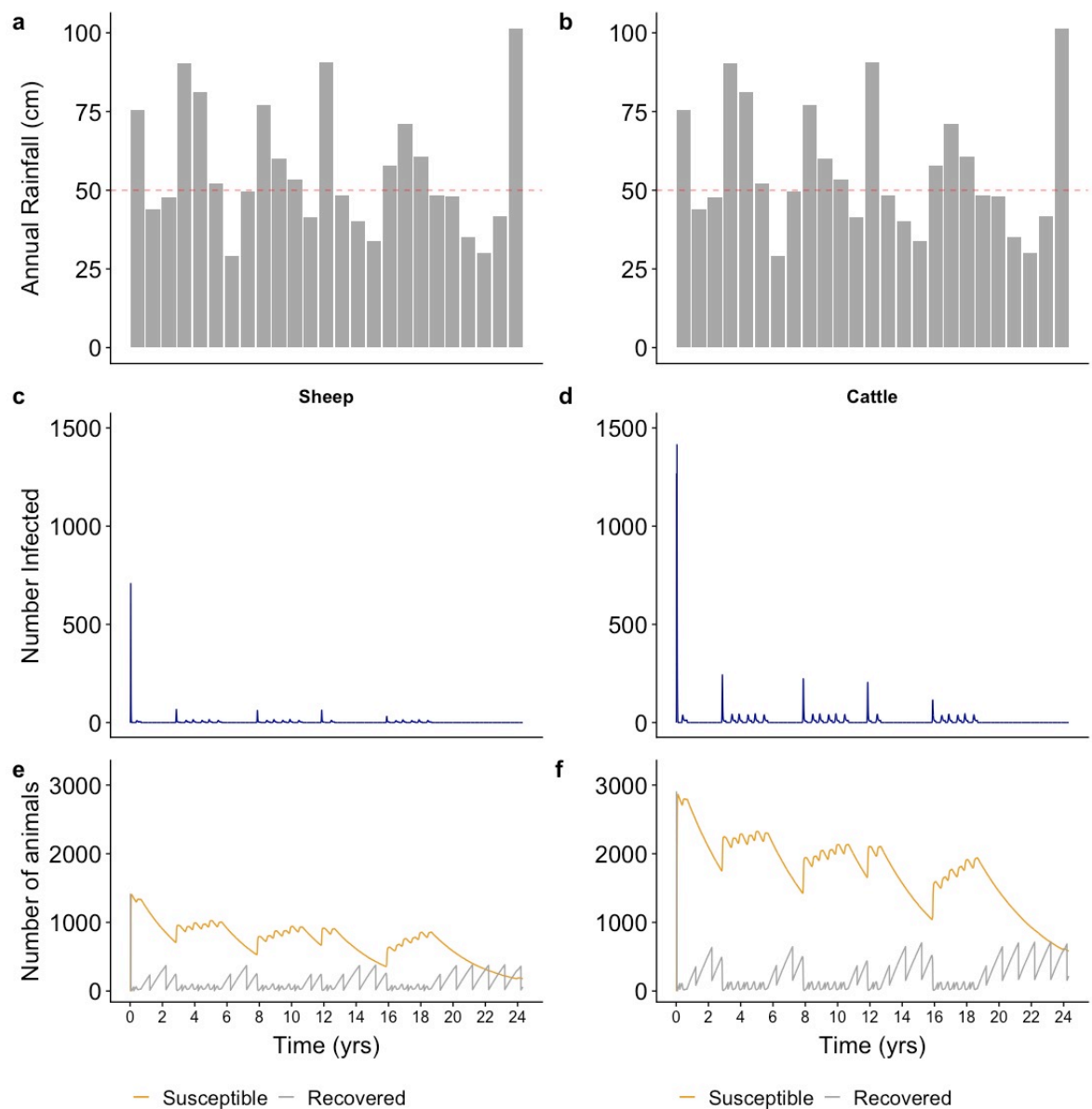


Figure 8.10 Transmission dynamics of Rift Valley fever virus in a population of sheep and cattle over a 24-year period when vaccinated with ChAdOx1 RVF. (a,b) Annual precipitation driving outbreaks. The red dashed line indicates the threshold for high levels of vector activity. (c,d) Number of infected (c) sheep and (d) cattle. (e,f) Number of susceptible (yellow) and recovered (grey) (e) sheep and (f) cattle. Results show the median of replicates of the model.

8.3.5. Assessing periodic vaccination with ChAdOx1 RVF

To further understand how best to implement a ChAdOx1 RVF vaccination strategy for RVF, transmission dynamics were assessed over the 24-year period with four re-vaccination scenarios: annual, every 3 years, every 5 years or every 8 years (Figure 8.11). These were picked considering the sporadic nature of outbreaks, lifespan of animals, willingness from farmers and feasibility. Results show the optimal strategy is vaccinating annually, though vaccinating every 3 years would reduce cumulative infected cattle and sheep by a significant amount compared to no vaccine at all ($p < 0.001$). Vaccinating every five years does reduce the cumulative infected cattle and sheep slightly whilst little reduction in cumulative infected livestock is seen if vaccinated every eight years.

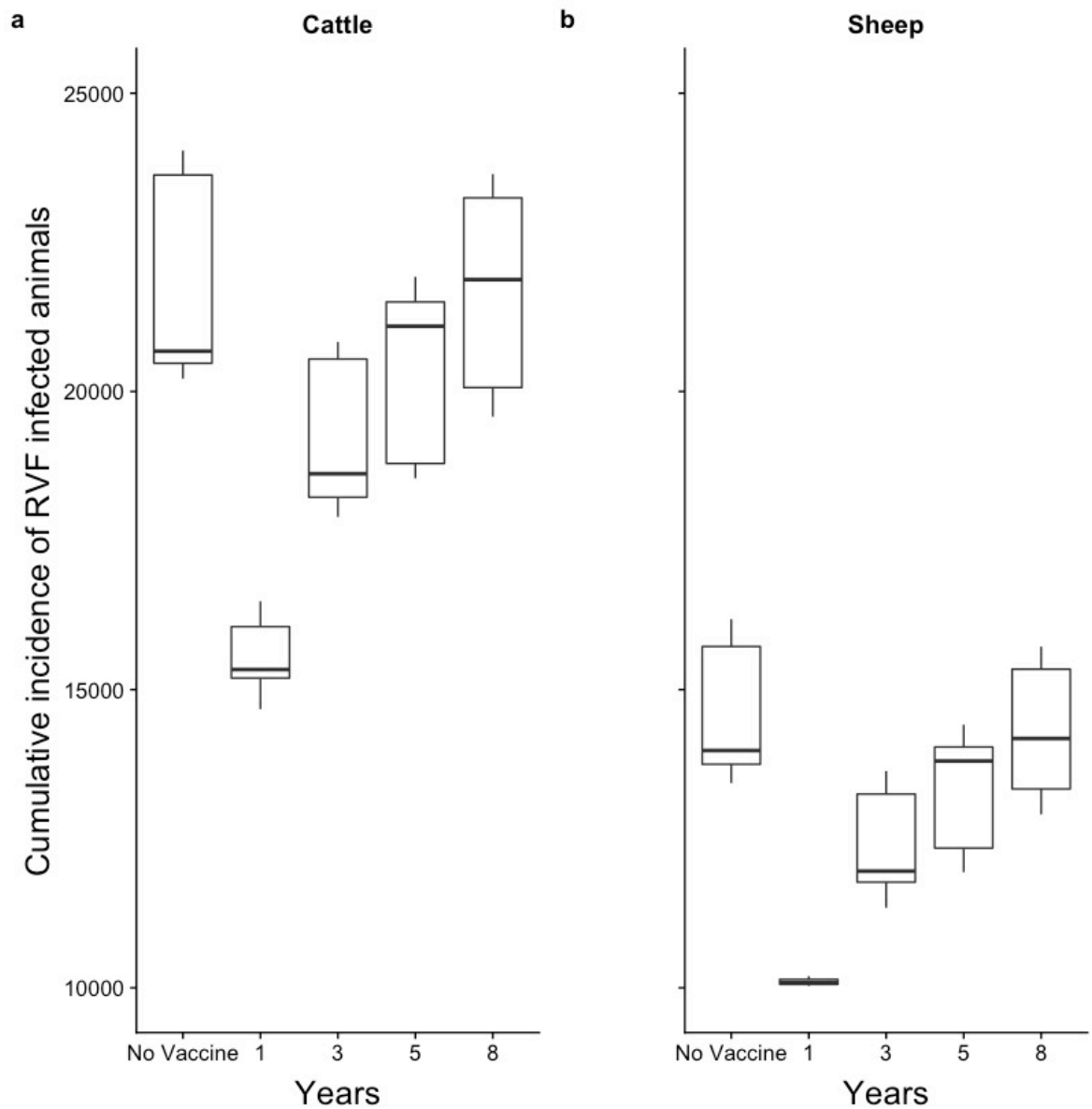


Figure 8.11 Effect re-vaccination has with ChAdOx1 RVF on cumulative incidence of infected cattle (a) and sheep (b) when vaccinating in dry season (Revaccination scenario, Table 8.3). Box and whiskers show the median and interquartile range of 100 replicates of the model.

8.4. Conclusions

This chapter presents a stochastic model for RVFV transmission dynamics that runs over a 24-year period. During this time period, multiple outbreaks associated with heavy persistent rainfall were generated in the model. This is in keeping with the conditions that have led to previous outbreaks in Kenya over the last nine decades (Murithi et al. 2010, Anyamba et al. 2001). The initial outbreak in the model was much higher than subsequent ones, because at this time the population of cattle and sheep would have been completely susceptible. Subsequent outbreaks resulted in numbers of infected animals similar to that reported in the literature during outbreaks (Clark et al. 2018). This model generated major outbreaks which occurred approximately every four years, coinciding with the life span of animals hence, when there are likely to be a larger number of susceptible animals in the herds. This pattern has been reported in Senegal with outbreaks in ruminant herds (Chitnis et al. 2013).

Smaller transient outbreaks followed larger outbreaks: the percentage of animals infected during these small outbreaks was very small (1%) when compared to the initial total population of animals on the farm. As these numbers are small and symptoms are non-specific it is possible these infections may go unnoticed as RVF and therefore may play a part in inter-epidemic period (IEP) transmission.

Vaccinating during the rainy season does not have as much impact as vaccinating during dry seasons irrespective of the efficacy of the vaccine. In this model the vaccination day was set 15 days after the rainy season started. The exact day of vaccination was crudely set but represented a reactive outbreak scenario, which also accounted for logistical issues such as acquiring the vaccine. It is worth noting here that other studies have modelled the process of vaccination differently; often vaccination runs over longer periods than a day (Gachohi et al. 2016, Métras et al. 2020). In the context of this present model with this number of animals

and on one farm, a single day would be enough to vaccinate all animals. To be able to make a realistic difference to the number of infected animals, a high efficacy vaccine would be needed for use during the rainy season. Results from this model support previous findings that delaying implementation of vaccination during an outbreak can lead to outbreaks that are of a similar magnitude to no vaccination at all (Chamchod et al. 2017). This is particularly important when considering the public health potential of this disease as well.

The effect of vaccinating in the dry season is much more pronounced at all vaccine efficacy levels. Although the aim is to have a high vaccine efficacy, a lower vaccine efficacy would have much more of an impact on reducing the cumulative incidence of infected cattle when vaccinated in the dry season than in the rainy season. In this situation the dry season vaccination date was set at day 200 which is 50 days after the first rainy season and 50 days before the second short rainy season, providing enough time for animals be vaccinated and mount an immune response before the next at-risk time point. Other studies have also shown that the cumulative incidence of infected ruminants is reduced when more animals are vaccinated before an outbreak occurs (Chamchod et al. 2017). This emphasises the need for better methods in predicting RVF outbreaks or having more formal routine vaccination strategies. These results support previous findings that control methods may need to be adapted based on the season and the situation (reducing endemicity or epidemicity) (Chitnis et al. 2013).

Unlike other models there was no effect on cumulative incidence of infected sheep when only cattle were vaccinated and vice versa (Gachohi et al. 2016). This may be due to the smaller numbers of animals used in this model.

In chapter 7, ChAdOx1 RVF was found to be a non-inferior vaccine for immunogenicity compared with the Smithburn vaccine. The model predicts that ChAdOx1 RVF vaccine significantly reduced incidence of RVFV in both sheep and cattle over the 24-year period compared to the Smithburn vaccine. ChAdOx1 RVF also met the vaccine efficacy requirements (50% or more) needed for an effective vaccination campaign as reported by previous models (Gachohi et al. 2016). Small outbreaks still occurred, but this can be explained by susceptible new-born animals which is in keeping with the recorded susceptibilities seen in young livestock. There are also other issues such as unique host response, failures with administering vaccines, human error which may play a part in small outbreaks occurring despite vaccination. The Smithburn vaccine was not efficacious nor able to control transmission dynamics of RVF, though this may have been due to the batch of vaccine used in the trial as outlined in chapter 7.

Annual ChAdOx1 RVF vaccination is the optimum strategy to reduced incidence of disease in livestock. However, various issues, including funding and assessment of risk, may mean that farmers would not necessarily do this. To mitigate outbreaks effectively, vaccinating every three years would be suitable; this would coincide with average lifespan of livestock species in Kenya and would mean herd immunity was maintained.

This model has been able to address the impact of vaccination on RVF transmission on a long timescale, across multiple outbreaks. The model has made numerous assumptions which require more research to refine outputs. Table 8.2 outlines the confidence in the parameters used in the model and where further research is needed to make model predictions more robust. In particular, more research is needed on assessment of biting rates and transovarial transmission. There are very few papers that directly investigate the role of transovarial transmission for RVF transmission and maintenance. The basis of RVF

transovarial transmission stems from one paper alone (Linthicum et al. 1985). Although a widely accepted maintenance role, it is understood that transovarial transmission alone cannot be the only mechanism for RVF IEP persistence (Manore and Beechler 2015). Lo Iacono et al. (2018) have modelled vector dynamics in relation to environmental limits (availability of waterbodies and varying temperature) demonstrating conditions needed for vector persistence, and hence, endemic transmission. Here, the authors emphasise frequency of fluctuations of water-bodies are needed for persistence of RVF in *Aedes* spp. as well as suitable livestock numbers. In the present model, transovarial transmission and the proportion of susceptible livestock drive transmission. Further investigations need to consider other mechanisms that may impact IEP transmission such as wildlife, conducive environments and other susceptible host species (Manore and Beechler 2015).

This model, like several others before (Gachohi et al. 2016, Métras et al. 2017) did not account for temperature which has been shown to have an effect on vector activity and, hence, RVFV transmission (Turell et al. 1985). Temperatures above 26°C can cause an increase in RVF transmission in *Aedes taeniorhynchus* and *Culex pipiens* mosquitoes (Turell et al. 1985). The contextual setting of Kenya means most areas do have temperatures above 26°C. However, certain areas in Machakos, including the Kapiti ranch can get quite cold at night, which may help account for why RVF outbreaks have not been recorded on the farm.

It is well known that vaccinating with the Smithburn vaccine should not be used whilst an outbreak is occurring due to the worries of genetic reassortment and recombination with a wildtype virus. This is a particular risk if needles are not changed between animals (Grobbelaar et al. 2011). The potential transmission of a vaccine isolate by vectors can also be an issue (Faburay et al. 2017). In this model vaccine transmission was not modelled, particularly as this is unlikely to be an issue for ChAdOx1 RVF.

This chapter has developed a theoretical model to assess impact of RVF vaccination on livestock. To assess the usefulness of the model, real life outbreak and vaccination data should be fitted to the model. Unfortunately, this was not available when the model was developed. This raises the importance of collecting this type of data whilst outbreaks are ongoing and making data rapidly available in the public domain as a critical need for public health action. This has been highlighted by international organisations following the west Africa Ebola outbreak and the ongoing COVID-19 outbreak (Whitty et al. 2015, Moorthy et al. 2020).

Due to time constraints a sensitivity analysis was not conducted, but this should be done to assess the uncertainty in the model outputs and how important each parameter is in determining the model outputs. To further assess the impact of RVF livestock vaccination, future research should consider what impact vaccination could have on spillover transmission to humans and the effect veterinary vaccination would have on public health. This has recently been accomplished by fitting outbreak data from the island of Mayotte to a compartmental model for RVFV, emphasising the importance of livestock vaccination on reducing disease risk in humans (Métras et al. 2020). Another model has also shown the cost-effectiveness of vaccinating livestock against RVF and the impact this has on public health (Kimani et al. 2016).

Spillover transmission is mainly associated with frequency of contact with infected blood and tissue of livestock. Less is known about the mechanisms of vector transmission to humans. Spillover events are rare and thus complex to model: understanding the abundance and prevalence in the reservoir, mechanisms of contact, susceptibility of human hosts and a conducive environment for this to take place. These methods of spillover can be described

by stochastic Poisson processes linking the animal disease dynamics and contact with humans (Lo Iacono et al. 2016). This additional analysis would aid considerably in informing appropriate control measures for zoonotic diseases, which could no doubt reduce the impact on animal and public health as well economically, especially if resources are shared between animal and public health organisations (Lo Iacono et al. 2016, Kimani et al. 2016). This type of research acts as a bridge between veterinary and public health disciplines sitting under a One Health framework. It has already been acknowledged that there are large gaps in modelling multi-host zoonotic diseases and particularly in assessing spillover (Lloyd-Smith et al. 2014). Much can be learnt and utilised in transmission models for zoonotic diseases from food safety research assessing risk of transmission from consumed animal meat to humans (Jordan et al. 1999).

This chapter assesses the effectiveness of livestock vaccination in reducing RVFV transmission: it emphasises the potential impact ChAdOx1 RVF vaccination could have on cattle and sheep herds on commercial farms in Kenya and suggests optimum re-vaccination strategies which will be helpful to local policymakers. The sporadic nature of RVF outbreaks begs the question of whether Kenyan farmers are willing to pay for vaccination against the disease and how often they would be prepared to vaccinate. A study in three counties of Kenya has shown farmers are willing to vaccinate but this depends on the price of vaccine and how many animals they have (Wanyoike et al. 2019). Other challenges to implement vaccination strategies are understood although not integrated into the model including the logistics of getting to RVF endemic areas which are often remote, acquiring vaccines, having a cold chain and systems (logistics and trained personnel) in place to make sure all animals are vaccinated.

9. Discussion

9.1. Principal Findings

RVF is a zoonotic disease characterized by sporadic outbreaks and, at the onset of this DPhil project, commercialized veterinary vaccines were not fit for purpose due to being contraindicated in pregnant animals, during outbreaks and not being DIVA compatible. The thesis chapters provide a sequential route from RVF seroprevalence surveillance in target species; set up, safety, immunogenicity and non-inferiority assessment of a novel RVF vaccine in the field; and investigating the impact of this vaccine. The principal findings are summarized based on the aims outlined in the introduction (chapter 1).

The systematic review (chapter 2) consolidated published literature on RVF seroprevalence in livestock, wildlife and humans in Africa. The principal findings from this chapter emphasized that small ruminants are the most at-risk species for RVF; these species have significantly higher RVF seroprevalence during outbreak periods than interepidemic periods. It is hypothesized that the population is made up of more naïve animals due to having shorter lifespans. Small ruminants, on average, do not have a lifespan that includes multiple outbreaks. Another significant finding from this chapter was the lack of RVF seroprevalence studies that assess wildlife, livestock and humans concurrently and hence spillover transmission. This has been identified as a problem across zoonotic disease surveillance: the epidemiology at the animal-human interface and associated pathways are needed to gain more understanding on disease transmission dynamics of these diseases (Lloyd-Smith et al. 2009, Vrbova et al. 2009, Halliday et al. 2012).

Both the systematic review (chapter 2) and the seroprevalence studies (chapter 3) confirm that livestock animals can seroconvert during interepidemic periods and although the maintenance of the virus is not fully understood it is likely they would play some role in virus transmission. This emphasises the role for veterinary vaccination against the virus,

specifically with a DIVA compliant vaccine. In some parts of Kenya, the role of vaccinating livestock species as a control measure is more evident than others. High risk areas such as Laikipia, confirmed in the seroprevalence study (chapter 3) and previously published papers (Evans et al. 2008, Munyua et al. 2016), are target counties that would benefit from vaccination campaigns as a preparedness strategy. In contrast, areas where no seroconversion was identified such as Kisumu should not be targeted for vaccination campaigns in the first instance but continued regular syndromic surveillance should remain in place (Thumbi et al. 2015). Furthermore, animal movements should be monitored in areas such as these which have little to no RVF activity to control incursions of the disease.

Chapter 4 showed that livestock species do have pre-existing antibodies to the ChAdOx1 (Y25) virus or at least a cross-reacting virus, but this does not impact on eliciting an immune response to the vaccine antigen. The chapter confirms ChAdOx1 is a suitable vaccine platform to use in livestock species and supports already identified results in the medical field (Antrobus et al. 2014, Wilkie et al. 2020). However, there is a question in the use of viral vector vaccines for multiple diseases. If the same platform is used, immunity to the vaccine vector could occur which may affect vaccine performance for other diseases. Ideally, in the future, a viral vector vaccine would be developed that could target multiple diseases.

A large component of this thesis (chapter 5-7) was the successful implementation and analysis of results from the first genetically engineered veterinary vaccine field trial conducted in Kenya: assessing non-inferiority of the ChAdOx1 RVF vaccine compared with the Smithburn vaccine in sheep, goats, cattle and camels. The trial confirmed ChAdOx1 RVF is a safe, DIVA compliant vaccine and preliminary results indicate that ChAdOx1 RVF is non-inferior to the Smithburn vaccine in all species.

Findings from the final chapter (chapter 8) indicate that ChAdOx1 RVF is a superior vaccine to control RVF transmission dynamics in sheep and cattle across multiple years. The optimum vaccination strategy would implement vaccination in the dry season and re-vaccinate annually, although vaccinating every 3 years also significantly reduces cumulative incidence of disease in sheep and cattle.

9.2. Strengths and limitations

This thesis, where possible, has used published guidelines to conduct and complete pieces of work. This has meant research has been transparent and standardised, providing as much detail as possible as to how studies were conducted. Table 9.1 records the guidelines used. An important consideration is the generalisability of the findings from the systematic review, seroprevalence studies and modelling work. RVFV transmission and hence seroprevalence in livestock species is a complex, context-dependent process which will be different at country, county, farm and individual animal level. The heterogeneity of study designs will also have an effect on associations. Due to the non-specific symptoms many cases will be missed unless testing is conducted; this is especially true for infected animals during interepidemic periods. Syndromic surveillance is therefore an important tool in disease preparedness and rapid response.

A significant outcome of this study was the successful collaborations with national regulators, external monitors and successfully running a multi-species field trial to vGCP standards (chapter 5). Following these guidelines means trial sponsors, monitors, the national regulators and the institutes involved with the grant could have confidence in how the trial was run both from an ethical/welfare perspective and in returning scientifically valid

results. This will be important to report when registering the ChAdOx1 RVF vaccine, as it will show regulators the standard to which the trial was carried out.

Table 9.1 Checklists used for studies in this thesis

Acronym	Title	Explanation	Chapters	Ref.
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses	Checklist for reporting systematic reviews	2	Moher et al. 2009
STROBE statement	Strengthening the reporting of observational studies in epidemiology	Checklist for reporting on epidemiological studies	2 and 3	Von Elm et al. 2007
ARRIVE	Animal Research: Reporting of <i>In Vivo</i> Experiments	Guidelines for reporting of research using animals to improve the design, analysis and reporting, maximizing information published	5, 6 and 7	Kilkenny et al. 2010
vGCP	Veterinary Good Clinical Practice	Guidelines for designing, conducting, monitoring, recording, analyzing and reporting clinical trials in animals	5, 6 and 7	European Medicines Agency (2000)
CONSORT	Consolidated Standards of Reporting Trials	Recommendations for reporting randomized trials: how the trial was designed, analysed and interpreted	5, 6 and 7	Piaggio et al. (2012)

The methodological choices for the seroprevalence chapter (chapter 3) were constrained as samples were taken opportunistically from surveillance systems and recruitment trials which had alternative primary aims that weren't specifically assessing RVF seroprevalence using randomized sampling. This also meant some data could not be captured. However, the opportunistic testing of the same camel herd over one month showed evidence of seroconversion within that time. There were worries this may have been due to mosquito mechanical transmission of the Smithburn vaccine virus as five camels had been vaccinated in that first month. The non-inferiority field trial in the four livestock species confirmed no signs of mechanical transmission so it is believed the camel IgG seroconversion to RVF was due to a natural outbreak. Interpretation of this finding should be made with caution as the Laikipia and Kapiti settings are different and certain stimuli such as mosquito abundance may play a role in why no mechanical transmission was seen. One paper has previously assessed vector transmission of the MP12 vaccine and no evidence of transmission was recorded (Miller et al. 2015). However, mechanical transmission of the Smithburn vaccine has not been assessed in the literature before and results from this trial support the lack of evidence of this.

The results showing ChAdOx1 RVF and Smithburn vaccines are safe in the livestock species are of paramount importance. Public engagement work I conducted prior to commencing the DPhil showed that the general public's biggest worry with genetically engineered vaccines was safety (Clark 2016). The only consideration here was the subjectivity of scoring in the safety assessment. In future, consensus training with trial veterinarians should be conducted prior to starting any trials.

As emphasised, the FRNT results for the non-inferiority trial are preliminary, as the tests used have not been formally validated. Confirmation of the tests using different RVF viruses

will increase confidence in results. Limitations of the non-inferiority protocol itself have already been addressed in chapter 7, specifically setting the non-inferiority margin. To better justify this margin would require more surveillance and monitoring of vaccinated herds to establish true effect sizes.

The model addressed questions relating to timings of vaccination and revaccination as well as predicting what could happen to RVF transmission dynamics over multiple years. Unfortunately, there were no available data to check the model, nor was a sensitivity analysis conducted due to time constraints. This is a significant limitation for this model and for many epidemic diseases. This emphasises the need for more monitoring and evaluation of veterinary vaccines in the field (Thomas et al. 2018). The ongoing field trial is assessing antibody duration for the animals vaccinated with Smithburn and ChAdOx1 RVF; these data could be added to the model to make predictions more robust on the impact of vaccine efficacy and immunogenicity on transmission dynamics.

9.3. Comparison with the state of knowledge prior to the work

The seroprevalence studies have built on previous studies assessing seroprevalence in Kenya during IEPs before 2016 (Labeaud et al. 2007, Lichoti et al. 2014, Lwande et al. 2015, Nanyingi et al. 2016). In chapter 3 I was able to assess RVF seroprevalence in livestock animals in subsequent years of 2017, 2018 and 2019. Conclusions from chapter 3 support findings from previous papers: seroconversion varies with location, sex and age. Previous studies have also identified areas where silent circulation during IEPs is taking place (Labeaud et al. 2007, Labeaud et al. 2008, Lichoti et al. 2014). The high rate of infection recorded in the goats in Laikipia may suggest ongoing virus transmission in the area. The lack of RVF seropositivity in Kisumu is supported by previous studies conducted in humans, it was hypothesised that this is because there is no endemic circulation in the area (Cook et

al. 2017). As the chapter 3 study only tested one or few farms in each county these results cannot be generalised to confirm risk levels reported for counties on published risk maps but does support risk levels for Laikipia and Kisumu (Munyua et al. 2016).

Prior to conducting this work, a number of candidate RVF vaccines were identified as DIVA compatible (Faburay et al. 2017). However, to my knowledge no study has tested this many livestock in terms of DIVA compatibility, and along with its additional beneficial characteristics as a vaccine, ChAdOx1 RVF is the superior vaccine in development. A number of novel RVF vaccine trials have been conducted in livestock including comparing novel vaccines with the Smithburn vaccine (von Teichman et al. 2011, Warimwe et al. 2016), but these were not conducted in the field nor were they powered to formally assess performance in comparison to licensed products (e.g. in a non-inferiority trial) (Nyundo et al. 2019).

This was the first-time anti-vector immunity was assessed in livestock animals, although this has been assessed in human populations before (Dudareva 2009, Bliss et al. 2017). The results show that pre-existing anti-vector immunity does not affect vaccine efficacy, but more research is needed to understand why pre-existing antibodies are present or if cross-reactivity is occurring.

9.4. Implications of the research

There is a role for the ChAdOx1 RVF as a veterinary vaccine for livestock in Kenya. However, realistically there are a number of hurdles to be overcome before such control measures could be put in place. Firstly, an important consideration is the interest and willingness from farmers to vaccinate, as well as support from government. Although studies

have shown farmers in the country are willing to vaccinate, the cost of vaccine would be critical, especially for farmers with larger numbers of livestock (Wanyoike et al. 2019). Due to the sporadic nature of the virus and its non-specific clinical signs, many farmers may decide it is not worth vaccinating. This would then become an issue for government where options may include subsidising or funding vaccination altogether or enforcing restrictions on livestock that are not vaccinated. One option is to maintain an emergency vaccine stockpile or use vaccine banks (Njenga et al. 2015). Chimpanzee adenovirus vaccines are suitable platforms to use for emergency stockpiling and production, especially for a disease which has a limited market (Ewer et al. 2017). For storage purposes ChAdOx1 RVF has previously shown stability under different conditions (25, 37 and 45°C) for long periods of time (Dulal et al. 2016).

The recent Global Burden of Animal Diseases (GBADs) programme which aims to develop systems to collect, validate and analyse the socio-economic impact of animal diseases and interventions will be a welcome initiative to aid in making decisions with regard to RVF vaccination and control mechanisms in Kenya and other at-risk countries (Thomas et al. 2018). Thomas et al. (2018) also highlight the importance of post-introduction studies for veterinary interventions to not only assess duration of effectiveness and safety but also logistics, distribution chains, implementation challenges, acceptability and value-for-money.

Vaccinating livestock against RVF is an output that would lead to economies of scale: agriculture is a huge part of the Kenyan economy; vaccinating would save animal lives, providing more agricultural outputs; aid in individual economies that use their livestock as investment (e.g. paying for school, medicines); and would protect against spillover transmission to humans (Marsh et al. 2016, Métras et al. 2020). Results have previously

shown that vaccinating cattle against East Coast fever virus provides significant direct effects including cattle health and productivity and indirect effects including household expenditure on education, food and medicine (Marsh et al. 2016). Governments should therefore consider subsidising or funding vaccination. The mathematical model generated in chapter 8 would be helpful for policy decision making as development for human vaccines is still ongoing,

A central concept in tackling such zoonotic pathogens with outbreak potential is disease preparedness rather than emergency response (Heymann and Dixon 2013). An optimal control strategy for RVF is pre-emptive vaccination in conjunction with national surveillance with buy-in from all stakeholders: government agencies to farmers (Oyas et al. 2018). RVF and many other veterinary and zoonotic diseases such as brucellosis, Schmallenberg virus and Wesselsbron virus have non-specific signs, and that is why there is a rational need to establish a national syndromic surveillance system in the country (Oyas et al. 2018). Successes of these surveillance systems have already been seen at a county level (Thumbi et al. 2015). Buy-in and direction will be needed from governmental authorities in Kenya such as the Department of Veterinary Services, Kenyan Wildlife Services and institutes such as the Kenya Medical Research Institutes (KEMRI) and the International Livestock Research Institute (ILRI) as well as farmers themselves. To be effective, surveillance systems and observational studies in general should be implemented and run in a standardised way following guidelines such as vGCP or STROBE (Table 9.1) so data can be analysed and reported on successfully. There are already several data collection platforms available that are being used for surveillance systems already and in innovative ways: Epicollect (Aanensen et al. 2009), REDcap (Harris et al. 2009) and the Open Data Kit (ODK) (Hartung et al. 2010). Investments into surveillance systems also need to address human motivation and behaviour to choose to report signs of a disease in the first place. This can be a complex

social issue and ultimately relies on meaningful understanding and community buy-in (Halliday et al. 2012).

The success of the RVF trial has enabled the ILRI Kapiti ranch to gain funding to become a centralised research organisation (CRO) to carry out veterinary vaccine clinical trials to the same standard. This facility should provide a valuable resource within the vaccine R&D sector in Africa and is supported by the harmonisation of regulatory procedures for registering new vaccines in the East Africa community supported by the Global Alliance for Livestock Veterinary Medicines (GALVmed) (Warimwe et al. 2018).

9.5. Future research directions

Collaborations across veterinary, medical and conservation biologists should be made to study disease dynamics between wildlife, livestock and humans. Surveillance including contact patterns should be run as longitudinal, sentinel studies, which as identified in the systematic review and elsewhere is underutilised in the context of infectious disease surveillance (Halliday et al. 2007, Clark et al. 2018). These studies could be used for the surveillance of multiple veterinary, zoonotic and human diseases. As has been recorded before, it is not just zoonotic diseases that can impact humans directly, but veterinary diseases as well, particularly in an economy that relies heavily on local agriculture (Marsh et al. 2016). Serological and contact results from these studies can be directly used in mathematical models to elucidate the effect of spillover transmission and to assess the impact vaccination would have across species. By conducting longitudinal studies on contact and transmission, risk assessments can also be conducted to identify risk factors and target control strategies towards these factors.

Further research is needed to confirm if livestock animals have been exposed to the ChAdOx1 (Y25) virus or if it is cross-reacting to another virus. This is particularly important to confirm efficacy of vaccines in these species. Sequences to the ChAdOx1 (Y25) virus should be compared with livestock adenoviruses to see if there is any homology and confirm if cross-reactivity is occurring.

The prospects for ChAdOx1 RVF as a One Health vaccine (i.e. one that can be used in animals and humans) looks increasingly promising. The formulation of a One Health vaccine will support economies of scale, lowering the cost of goods for farmers and government. This study is part of a larger research programme leading on the creation of a One Health RVF vaccine where the veterinary and medical vaccine use an identical master seed and the same manufacturing process so that only downstream processing and deployment will be distinct for veterinary and medical purposes. Other diseases such as human and bovine respiratory syncytial virus also have a potential viral-vectored vaccine in development (Taylor et al. 2015). Human clinical trials are now under way for a ChAdOx1 MERS-CoV vaccine (NCT04170829) which has already been successfully tested in camels (Alharbi et al. 2019). In a medical context the ChAdOx1 platform is currently being tested as a candidate vaccine for the COVID-19 coronavirus which was declared a pandemic by the World Health Organisation on 11th March 2020. Clinical trials using the ChAdOx1 platform are due to start late spring 2020 (Oxford Vaccine Group, 2020).

In order to enhance vaccine development and registration it will be important to engage with regulators across different fields as early in the process as possible: this includes veterinary, medical and food regulators. In order to facilitate this, organisations such as the OIE, WHO, international funders and network groups like the International Veterinary Vaccinology Network should support interactions through workshops and drafting policy.

To understand vaccine efficacy for protection against viraemia and whether sterile immunity is achieved, a vaccine trial would need to be conducted in a high-risk area (for example Laikipia) with RVF currently circulating. This is an obvious challenge as it is difficult to determine if RVF is actively circulating during an IEP. Similar issues are reported with other diseases such as the Ebola virus. This is why when outbreaks do occur and a new vaccine is used as a control measure, systems need to be in place to collect as much data (and samples where possible) in a standardised way that can be used for analysis.

Further research from the field trial will establish the duration of antibodies elicited by the ChAdOx1 RVF after one year and hence provide additional information on the cost-effectiveness of the vaccine. Further research is needed to establish the dosing of ChAdOx1 RVF, specifically for cattle and camels. In this study all four species were given the same dose of ChAdOx1 RVF due to successes of using the same dose seen in the previous challenge trials (Warimwe et al. 2016, Stedman et al. 2019).

It is well recorded that veterinary vaccine trials are not as stringently regulated and hence run to the same standard as human vaccine trials (Knight-Jones et al. 2014, Thomas et al. 2018). By conducting the non-inferiority trial to this level, we can say that our results were produced through standardized, validated, transparent mechanisms providing strength in the measurable scientific outcomes. These data can be used for getting the vaccine registered and both veterinary and human health regulators will find this useful. It also provides added support for the vaccine platform itself being used in other countries. We should not forget that this vaccine is classed as a GMO which requires additional levels of review to conduct clinical trials and close collaboration with regulators and monitoring teams. This trial has shown huge support and interest from the local regulators and government officials which has led to a collaboration where discussions can be had openly, and decisions can be made

based on scientific rationale. All of this will help in the next stages of creating a dossier for vaccine registration for the veterinary product and can be used as supporting material for future medical clinical trials, in the overarching aim of creating a One Health vaccine.

This thesis set out to determine the potential role of veterinary vaccination in the control of RVF in Kenya. The thesis has assessed this question from a systems ‘vaccinologist’ perspective, covering research from the lab, field and mathematical modelling. In 2016 I attended the European Veterinary Vaccinology workshop in Ghent hosted by the UK Veterinary Vaccinology Network and two EU Horizon 2020 projects, Paragone and SAPHIR. Here, Dr Catherine Charreyre, Boehringer Ingelheim described the key role of a vaccinologist as *‘a polyvalent expert on research, regulatory requirement, project management, clinical and analytical outcomes for vaccine development’* (taken from C.Charreyre presentation ‘Development of Veterinary Vaccines: Gaps and Requirements’. 9th May 2016). In this thesis I have covered many different elements of assessing the role of RVF veterinary vaccination in Kenya. I have confirmed the need for active surveillance across wildlife, livestock and humans to identify areas that need RVF vaccination; established that ChAdOx1 RVF vaccine is a non-inferior candidate vaccine for RVF, showing successful results for immunogenicity, safety and DIVA capabilities and overcoming issues reported with commercialised veterinary vaccines at present; and suggested that vaccinating annually or every 3 years during the dry season is the optimum control strategy to alleviate cumulative incidence of the disease. Until a human RVF vaccine is available, the role of veterinary vaccination for the control of RVF in Kenya is paramount.

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Appendices

Appendix A2.1 PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	44
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	See Clark et al. 2018
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	45
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	45
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	46
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	47
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	46
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	46
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	46-47

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	49
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	49
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	49
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	49
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	49

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	49
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	-
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	50
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	52-63
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	62-63
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	52-63

Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	52-63
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	62-63
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	-
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	64-66
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	67-68
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	69-70
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	See Clark et al. 2018

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:1.1371/journal.pmed1000097

Appendix A2.2 Reported seroprevalence of Rift Valley fever virus in livestock, wildlife and humans in Africa, 1968-2016

Country	Year	Camels	Goats	Sheep	Cattle	Humans	Wildlife	Ref
Botswana	2010	-	-	-	5.7	-	12.7	Jori et al 2015
Burkina Faso	1987	-	-	15.8	-	-	-	Gonzalez et al 1992
Burkina Faso	2005	-	17.5	20	22.5	-	-	Boussini et al 2014
Cameroon	2013	-	2.6	2.6	10	-	-	Rissmann et al 2017
Cameroon	2014	-	4.9	4.9	16.3	-	-	Rissmann et al 2017
Central African Republic	1992	-	-	-	8	-	-	Guilherme et al 1996
Central African Republic	2010	-	5	12.9	7.8	16.7	-	Nakoune et al 2016
Chad	2002	-	8	10.7	4	-	-	Ringot et al 2004
Comoros	2009	-	33.5	39	30.6	-	-	Roger et al 2011
Comoros	2010	-	7.85	-	7.85	-	-	Roger et al 2014
Comoros	2011	-	-	-	-	10.7	-	Dellagi et al 2016
Comoros	2011	-	27.6	-	27.6	-	-	Roger et al 2014
Djibouti	2010	-	-	-	-	2.2	-	Andayi et al 2014
Egypt	1984	-	-	6	-	-	-	Allam et al 1986
Egypt	1986	-	-	1.2	-	-	-	Botros et al 1988
Egypt	1989	-	-	-	-	4	-	Corwin et al 1993
Egypt	1989	-	-	-	-	4	-	Corwin et al 1992
Egypt	1991	-	-	-	-	15	-	Corwin et al 1993
Egypt	1993	-	-	-	-	2	-	Abu-Elyazeed et al 1996
Egypt	1993	-	-	-	-	12	-	Centers for Disease C 1994
Egypt	1993	-	-	-	-	8.4	-	Centers for Disease C 1994
Egypt	1993	-	-	-	-	77	-	Arthur et al 1993
Egypt	1997	-	-	100	100	-	-	Abd el-Rahim et al 1999
Egypt	1999	-	-	-	-	7.95	-	El E et al 2001
Egypt	2000	-	-	-	-	-	29.33	Youssef et al 2001
Egypt	2009	-	-	-	1	-	-	Horton et al 2014
Egypt	2013	-	-	-	50.7	-	-	Mroz et al 2017 ^a
Egypt	2014	3.17	0	0.46	-	-	-	Mroz et al 2017 ^b
Gabon	2005	-	-	-	-	3.3	-	Pourrut et al 2010
Gabon	2014	-	4.72	8.42	-	-	-	Maganga et al 2017
Gambia	1988	-	38	41	57	-	-	Ksiazek et al 1989
Ivory Coast	2012	-	0	3.9	3.9	-	-	Kanoute et al 2017
Kenya	1968	-	-	-	81	-	-	Davies et al 1975
Kenya	1987	-	-	-	-	2.8	-	Morrill et al 1991

Country	Year	Camels	Goats	Sheep	Cattle	Humans	Wildlife	Ref
Kenya	1987	-	-	-	-	-	0	Fischer-Tenhagen et al 2000
Kenya	1989	-	3.5	3.5	33.7	-	-	Kilelu et al 1993
Kenya	1994	-	-	-	-	19.1	-	LaBeaud et al 2007
Kenya	1996	-	-	-	-	0	-	LaBeaud et al 2007
Kenya	1996	-	-	-	-	3	-	LaBeaud et al 2007
Kenya	1997	-	-	-	-	0.9	-	LaBeaud et al 2007
Kenya	1997	-	-	-	-	1	-	LaBeaud et al 2007
Kenya	1997	-	-	-	-	15	-	Woods et al 2002
Kenya	1998	-	-	-	-	0	-	LaBeaud et al 2007
Kenya	1999	-	-	-	-	-	15.6	Evans et al 2008
Kenya	1999	-	-	-	-	-	6	Evans et al 2008
Kenya	1999	-	-	-	-	-	2.5	Evans et al 2008
Kenya	1999	-	-	-	-	-	32.6	Evans et al 2008
Kenya	1999	-	-	-	-	-	1	Evans et al 2008
Kenya	1999	-	-	-	-	-	62.5	Evans et al 2008
Kenya	1999	-	-	-	-	-	20	Evans et al 2008
Kenya	1999	-	-	-	-	-	10.9	Evans et al 2008
Kenya	1999	-	-	-	-	-	50	Evans et al 2008
Kenya	1999	-	-	-	-	-	87.5	Evans et al 2008
Kenya	2000	7.2	-	-	-	-	-	Britch et al 2013
Kenya	2004	-	-	-	-	0.7	-	Mease et al 2011
Kenya	2005	-	3	18	-	-	-	Rostal et al 2010
Kenya	2006	-	-	-	-	23	-	Anyangu et al 2010
Kenya	2006	-	-	-	-	13	-	LaBeaud et al 2008
Kenya	2006	-	-	-	-	26	-	Nguku et al 2010
Kenya	2006	-	-	-	-	18	-	Muiruri et al 2015
Kenya	2007	57.1	-	-	-	-	-	Britch et al 2013
Kenya	2007	-	-	-	-	4.5	-	Ochieng et al 2015
Kenya	2008	-	-	-	0	-	-	Lwande et al 2015
Kenya	2008	-	-	-	0	-	17.4	Lwande et al 2015
Kenya	2009	-	-	-	0	-	9.6	Lwande et al 2015
Kenya	2009	-	-	-	-	23	-	LaBeaud et al 2011
Kenya	2009	-	-	-	-	19.5	-	Tigoi et al 2015
Kenya	2009	-	-	-	-	1.8	-	Grossi-Soyster et al 2017
Kenya	2010	-	-	-	-	0.8	-	Cook et al 2017
Kenya	2010	-	-	-	-	2.5	-	Cook et al 2017
Kenya	2010	-	-	-	0.5	1.4	-	Fevre et al 2017
Kenya	2010	-	-	-	-	15.7	-	Gray et al 2015
Kenya	2010	-	-	-	0	-	0	Lwande et al 2015
Kenya	2010	-	69.6	69.6	-	-	-	Lichoti et al 2014
Kenya	2011	-	-	-	-	15	-	LaBeaud et al 2015
Kenya	2011	-	-	-	0	-	17.7	Lwande et al 2015
Kenya	2012	-	-	-	0	-	25	Lwande et al 2015
Kenya	2012	-	-	-	13.1	-	-	Owange et al 2014
Kenya	2013	-	25.8	32.2	33.3	-	-	Nanyingi et al 2017
Kenya	2013	-	-	-	0	-	0	Lwande et al 2015

Country	Year	Camels	Goats	Sheep	Cattle	Humans	Wildlife	Ref
Kenya	2014	-	-	-	0	-	11.6	Lwande et al 2015
Madagascar	1990	-	-	-	29.6	5.4	-	Morvan et al 1991
Madagascar	2005	-	24.7	24.7	25.8	-	-	Jeanmaire et al 2011
Madagascar	2009	-	-	-	28	-	-	Chevalier et al 2011
Madagascar	2010	-	-	-	-	0.3	-	Schwarx et al 2012
Madagascar	2010	-	-	-	-	6.3	-	Gray et al 2015
Mauritania	1993	-	18.72	18.72	-	-	-	Zeller et al 1995
Mauritania	1998	-	16.3	34.8	-	24.4	-	Nabeth et al 2001
Mauritania	2003	-	50	16	-	-	-	Faye et al 2007
Mauritania	2010	45	69	69	13	-	-	Jacket et al 2013
Mauritania	2012	32	3.8	3.8	15.4	-	-	Rissmann et al 2017
Mauritania	2015	-	-	-	-	30.97	-	Bob et al 2017
Mayotte	2004	-	-	-	22.66	-	-	Cetre-Sossah et al 2012
Mayotte	2005	-	-	-	3.1	-	-	Cetre-Sossah et al 2012
Mayotte	2006	-	-	-	12.31	-	-	Cetre-Sossah et al 2012
Mayotte	2007	-	-	-	30.95	-	-	Cetre-Sossah et al 2012
Mayotte	2007	-	-	-	37	-	-	Cetre-Sossah et al 2012
Mayotte	2007	-	13.79	-	-	-	-	Cetre-Sossah et al 2012
Mayotte	2007	-	-	-	10.6	-	-	Cetre-Sossah et al 2012
Mayotte	2010	-	22.4	22.4	26.8	4.1	-	Lernout et al 2011
Morocco	2009	15	-	-	-	-	-	El-Harrak et al 2011
Mozambique	1981	-	-	-	-	2	-	Niklasson et al 1987
Mozambique	2007	-	21.2	35.8	-	-	-	Fafetine et al 2013
Mozambique	2010	-	11.6	9.2	-	-	-	Fafetine et al 2013
Mozambique	2010	-	-	-	36.9	-	-	Lagerqvist et al 2013
Mozambique	2012	-	-	-	-	1.3	-	Gudo et al 2016 ^a
Mozambique	2013	-	-	-	-	5	-	Gudo et al 2016 ^b
Mozambique	2013	-	25.1	44.2	-	-	-	Blomstrom et al 2016
Mozambique	2014	-	55.7	55.7	-	-	-	Fafetine et al 2014
Namibia	1983	-	-	-	-	2	-	Joubert et al 1985
Namibia	1987	-	-	-	-	-	0	Fischer-Tenhagen et al 2000
Niger	1984	12.8	9.33	13.86	12.64	-	-	Mariner et al 1995
Niger	1985	12.8	9.33	13.86	12.64	-	-	Mariner et al 1995

Country	Year	Camels	Goats	Sheep	Cattle	Humans	Wildlife	Ref
Nigeria	1981	3.13	0.98	6.67	2.85	-	-	Ezeifeke et al 1982
Nigeria	1985	-	-	-	-	14.8	-	Olaleye et al 1996 ^a
Nigeria	1986	3.3	10.4	18.7	10.2	-	-	Olaleye et al 1996 ^b
Nigeria	1989	-	-	-	-	6.7	-	Olaleye et al 1996 ^a
Nigeria	2011	-	-	-	-	14.1	-	Bukbuk et al 2014
Rwanda	2012	-	-	-	16.8	-	-	Umuhoza et al 2017
Senegal	1988	-	21.77	21.77	-	-	-	Thiongane et al 1991
Senegal	1988	-	5	0	-	-	-	Ksiazek et al 1989
Senegal	1988	-	12.2	9.8	12.2	-	-	Clements et al 2007
Senegal	1989	-	-	30.1	-	22.3	-	Wilson et al 1994
Senegal	1991	-	4.6	4.6	5.8	-	-	Zeller et al 1997
Senegal	1995	-	40	40	-	-	-	Thonnon et al 1999
Senegal	1996	-	-	-	-	-	3.8	Gora et al 2000
Senegal	1998	-	-	-	-	5.2	-	Marrama et al 2005
Senegal	2003	-	5.4	5.4	-	-	-	Chevalier et al 2003
Senegal	2004	-	5.3	5.3	-	-	-	Chevalier et al 2009
Sierra Leone	2007	-	-	-	-	1.80	-	O’Hearne et al 2016
Somalia	2005	6.67	6.67	6.67	6.67	-	-	Soumare et al 2007
South Africa	1980	-	-	-	-	12	-	Swanepoel et al 1985
South Africa	1986	-	-	-	-	-	23	Pretorius et al 1997
South Africa	1987	-	-	-	-	-	0	Fischer-Tenhagen et al 2000
South Africa	1987	-	-	-	-	-	0	Fischer-Tenhagen et al 2000
South Africa	1993	-	-	-	-	-	0	BJ 1997
South Africa	2000	-	-	-	-	-	32	LaBeaud et al 2011
South Africa	2002	-	-	-	-	-	30	LaBeaud et al 2011
South Africa	2003	-	-	-	-	-	18	LaBeaud et al 2011
South Africa	2003	-	-	-	-	-	6.1	Fagbo et al 2014
South Africa	2004	-	-	-	-	-	19	LaBeaud et al 2011
South Africa	2005	-	-	-	-	-	14	LaBeaud et al 2011
South Africa	2007	-	-	-	-	-	49	Miller et al 2011
South Africa	2008	-	-	-	-	15	-	Archer et al 2013
South Africa	2008	-	-	2.73	35	-	-	Mapaco et al 2012
South Africa	2008	-	-	0.73	13.7	-	-	Mapaco et al 2012
South Africa	2008	-	-	-	-	15	-	Archer et al 2011
South Africa	2010	-	-	56.8	51.1	-	-	Odendaal et al 2014
Sudan	1979	7.9	22	34.3	33.2	-	-	Eisa M 1984
Sudan	1986	-	-	-	-	2.3	-	Woodruff et al 1988
Sudan	1989	-	-	-	-	23	-	Watts et al 1994

Country	Year	Camels	Goats	Sheep	Cattle	Humans	Wildlife	Ref
Sudan	2007	-	-	-	-	0.42	-	Hassanain et al 2010
Sudan	2014	9.6	-	-	-	-	-	Abdallah et al 2015
Tanzania	2004	-	-	-	-	4	-	Swai et al 2009
Tanzania	2007	-	-	-	-	5.2	-	Heinrich et al 2012
Tanzania	2010	27.5	-	-	-	-	-	Swai et al 2015
Tanzania	2011	-	11.86	11.37	11.03	-	-	Sumaye et al 2013
Tanzania	2011	-	4.7	12.5	-	-	-	Kifaro et al 2014
Tanzania	2012	-	-	-	-	11.7	-	Sumaye et al 2015
Tanzania	2013	-	21.9	29.7	27.8	-	-	Sindato et al 2015
Tanzania	2014	-	9.4	6.7	-	-	-	Wensman et al 2015
Tunisia	2006	-	0	0	-	-	-	Ayari-Fakhfakh et al 2011
Tunisia	2014	-	-	-	-	7.8	-	Bosworth et al 2016
Tunisia	2016	0	-	-	-	-	-	Hassine et al 2017
Uganda	2009	-	9.8	-	-	-	-	Magona et al 2013
Western Sahara	2008	0.97	0.97	0.97	-	-	-	Di Nardo et al 2014
Zambia	1986	-	-	-	22	-	-	Davies et al 1992
Zambia	1987	-	-	-	14	-	-	Ghirotti et al 1991
Zimbabwe	1989	-	-	-	-	-	6.28	Anderson et al 1998
Zimbabwe	1989	-	-	-	-	-	0.35	Anderson et al 1998
Zimbabwe	1989	-	-	-	-	-	0.12	Anderson et al 1998
Zimbabwe	1989	-	-	-	-	-	4.47	Anderson et al 1998
Zimbabwe	1989	-	-	-	-	-	8.3	Anderson et al 1998
Zimbabwe	1989	-	-	-	-	-	14.55	Anderson et al 1998
Zimbabwe	2008	-	-	-	12.1	-	5.3	Caron et al 2013

Appendix A5.1 Example of Field Trial Gantt Chart

		Mar-19										APRIL 2019																														MAY 2019									
	Day	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8		
	Female Sheep Study Day					-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
	Male Sheep Study Day					-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28			
Clinical examination	before day -15	S1	S1	S2	S2															S1	S2																														
Administration of treatment (IVP, CV, CP)	0																			S1	S2																														
Rectal temperature	-14, 0, 1, 3, 5, 7, 14, 28					S1	S2													S1	S1&2	S2	S1	S2	S1	S2	S1	S2																		S1	S2				
Clinical observation (vet)	-14, 0, 1, 3, 5, 7, 14, 28					S1	S2													S1	S1&2	S2	S1	S2	S1	S2	S1	S2																		S1	S2				
Injection site observation	0, 1, 3, 5, 7, 14, 28																			S1	S1&2	S2	S1	S2	S1	S2	S1	S2																			S1	S2			
Blood collection (Serum)	-14, -1, 14, 28, M3, M6, M9, M12					S1	S2													S1	S2																										S1	S2			
Blood collection (Vaccine viraemia)	-1, 1, 3, 5, 7, 14, 28																			S1	S2	S1	S2	S1	S2	S1	S2	S1	S2																		S1	S2			
Blood collection (Plasma)	-1, 7, 14, 28																			S1	S2							S1	S2																		S1	S2			
Blood collection (PMBC)	-1, 14																			S1	S2																											S1	S2		
Nasal swab collection	-1, 1, 3, 5, 7, 14, 28																			S1	S2	S1	S2	S1	S2	S1	S2	S1	S2																		S1	S2			
Rectal swab collection	-1, 1, 3, 5, 7, 14, 28																			S1	S2	S1	S2	S1	S2	S1	S2	S1	S2																		S1	S2			
PBMCs																				S1	S2																											S1	S2		

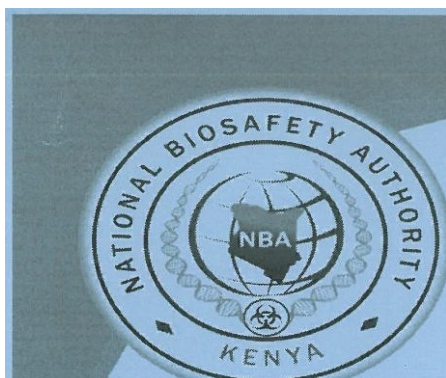
Appendix A5.2 Consort statement of non-inferiority and equivalence trials

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	<u>How participants were allocated to interventions</u> (e.g., "random allocation", "randomized", or "randomly assigned"), <i>specifying that the trial is a non-inferiority or equivalence trial.</i>	115
<i>INTRODUCTION</i> Background	2	Scientific background and explanation of rationale, <i>including the rationale for using a non-inferiority or equivalence design.</i>	116-119
<i>METHODS</i> Participants	3	<u>Eligibility criteria for participants</u> (<i>detailing whether participants in the non-inferiority or equivalence trial are similar to those in any trial(s) that established efficacy of the reference treatment</i>) and the <u>settings and locations where the data were collected.</u>	129, 128, 141
Interventions	4	<u>Precise details of the interventions intended for each group detailing whether the reference treatment in the non-inferiority or equivalence trial is identical (or very similar) to that in any trial(s) that established efficacy, and how and when they were actually administered.</u>	118, 119, 123, 124, 131, 147, 149
Objectives	5	<u>Specific objectives and hypotheses, including the hypothesis concerning non-inferiority or equivalence.</u>	123, 193
Outcomes	6	<u>Clearly defined primary and secondary outcome measures detailing whether the outcomes in the non-inferiority or equivalence trial are identical (or very similar) to those in any trial(s) that established efficacy of the reference treatment</u> and, when applicable, any <u>methods used to enhance the quality of measurements</u> (e.g., multiple observations, training of assessors).	123, 124
Sample size	7	<u>How sample size was determined</u> <i>detailing whether it was calculated using a non-inferiority or equivalence criterion and specifying the margin of equivalence with the</i>	127, 193

		<i>rationale for its choice. When applicable, <u>explanation of any interim analyses and stopping rules</u> (and whether related to a non-inferiority or equivalence hypothesis).</i>	
Randomization -- Sequence generation	8	<u>Method used to generate the random allocation sequence, including details of any restrictions</u> (e.g., blocking, stratification)	127
Randomization -- Allocation concealment	9	<u>Method used to implement the random allocation sequence</u> (e.g., numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	127
Randomization -- Implementation	10	<u>Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.</u>	127
Blinding (masking)	11	<u>Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment.</u> If done, <u>how the success of blinding was evaluated.</u>	127
Statistical methods	12	<u>Statistical methods used to compare groups for primary outcome(s), specifying whether a one or two-sided confidence interval approach was used. Methods for additional analyses, such as subgroup analyses and adjusted analyses.</u>	193
RESULTS Participant flow	13	<u>Flow of participants through each stage</u> (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. <u>Describe protocol deviations from study as planned, together with reasons.</u>	123-124, 151
Recruitment	14	<u>Dates defining the periods of recruitment and follow-up.</u>	124, 149
Baseline data	15	<u>Baseline demographic and clinical characteristics of each group.</u>	129, 163
Numbers analyzed	16	<u>Number of participants (denominator) in each group included in each analysis and whether the analysis was “intention-to-treat” and/or alternative analyses were conducted.</u> State the results in absolute numbers when feasible (e.g., 10/20, not 50%).	196-199
Outcomes and estimation	17	<u>For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision</u> (e.g., 95% confidence interval). <i>For the outcome(s) for which non-inferiority or equivalence is hypothesized, a figure showing confidence intervals and margins of equivalence may be useful.</i>	196-200

Ancillary analyses	18	<u>Address multiplicity by reporting any other analyses performed</u> , including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	194-195 163-183
Adverse events	19	<u>All important adverse events or side effects in each intervention group.</u>	163-183
<i>DISCUSSION</i> Interpretation	20	<u>Interpretation of the results</u> , taking into account the <i>non-inferiority or equivalence hypothesis and any other</i> study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	201-205
Generalizability	21	<u>Generalizability (external validity) of the trial findings.</u>	204-205
Overall evidence	22	<u>General interpretation of the results in the context of current evidence.</u>	201-205

Appendix A5.3 NBA Approval letter



NATIONAL BIOSAFETY AUTHORITY

COMMISSION FOR UNIVERSITY EDUCATION BUILDING
REDHILL ROAD, OFF FLIMURU ROAD

P. O. Box 28251 - 00100, Nairobi. | Tel: +254 20 267 8667

Email: info@biosafetykenya.go.ke | Website: www.biosafetykenya.go.ke

When replying please quote:

Our Ref: NBA/GMO/Co9/71

25th November 2016

Director General,
International Livestock Research Institute (ILRI),
P. O. Box 30709-00200,
Nairobi, Kenya.

Attn: Dr. Vish Nene,
Chairman, ILRI Institutional Biosafety Committee.

Dear *rJ_e,v-A-- I*

APPROVAL DOCUMENT FOR THE APPLICATION TO CONDUCT CONFINED FIELD TRIAL OF A NOVEL RIFT VALLEY FEVER VACCINE IN SHEEP, GOATS, CATTLE AND CAMELS

The above subject matter refers.

Following your application to the Authority seeking authorization to conduct a confined field trial of a novel Rift Valley Fever vaccine in sheep, goats, cattle and camels at Kapiti Ranch, we wish to advise you that the application has been reviewed and is hereby approved.

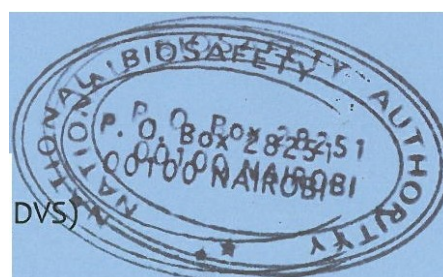
Attached herein, please find the approval document reference number **NBA/GMO/CO9/18/27** and the conditions attached to the approval.

We look forward to a harmonious working relationship with you as the project commences.

Yours _____/

Willy K. Tonui

WILLY K. TONU, PhD, RBP
CHIEF EXECUTIVE OFFICER



Copy to: Dr. Kisa JumaNgeiywa
Ag. Director, Department of Veterinary Services (
Veterinary Research Laboratories, Kabete
Private Bag 00625
.Kangemi....Nairobi

FOURTH SCHEDULE

(r.9)



NATIONAL BIOSAFETY AUTHORITY

**APPROVAL TO CONDUCT CONFINED FIELD TRIAL OF A NOVEL RIFT VALLEY FEVER
VACCINE IN SHEEP, GOATS, CATTLE AND CAMELS.**

APPROVAL NUMBER:	DATE OF ISSUE : 25 th November, 2016
NBA/GMO/Co9/18/27	VALID UPTO : 25 th November, 2021
In accordance with regulation 9 of the Biosafety (Contained Use) Regulations, of the Biosafety Act 2009, I hereby grant the approval to undertake contained use activity of the genetically modified organism herein stated in the research institution mentioned in this approval.	
Name of the Applicant/ Research Institution	International Livestock Research Institute (ILRI)
Specification of the genetically modified organism	A recombinant DIVA vaccine (ChAdOx1-Gn,-Gc) composed of a replication- deficient simian adenovirus vector encoding RVF virus envelope glycoproteins.
Quantity approved	A single batch of the ChAdOx1-Gn-Gc vaccine will be used in this project. Both male and female animals will be used in this study. A total of 720 animals will be used i.e. Cattle - 180, Sheep -180, Goats-180 and Camels-180.
Specification of the genetic modification	Vaccine (ChAdOx1-GnGc) was prepared by Gateway® recombination between the ChdOx1 vector and an entry plasmid having coding sequence for RVF virus envelope glycoproteins. After viral rescue the vaccine was propagated in HEK293 and subsequently purified by Caesium Chloride (CsCl) gradient ultracentrifugation.
Risk category	Low
Purpose of the use:	The purpose of this confined field trial is to evaluate ChAdOx1-Gn-Gcrecombinant vaccine in a confined field trial at Kapiti Ranch, Machakos County, to further assess its safety, and immunogenicity among sheep, goats, cattle and drom dary camels in Kenya.
This approval is granted subject to the following conditions:-	
1. Import permit for ChAdOx1-Gn-Gc must be obtained from DVS who will ensure that the vaccine is well packaged and securely transported from the airport to ILRI. 2. The Confined Field Trial site must be inspected by NBA and DVS before the	

commencement of the trial. 3. Provide operational manuals/SOPs on biosafety and biosecurity measures before commencement of trials. 4. A representative from NBA and DYS to be present during the vaccination and the disposal of the unused vaccine as well as the destruction of all livestock samples following the termination of the trial. 5. A detailed schedule of activities and the experimental design must be provided both to NBA and DYS before commencement of the trial to aid in monitoring purposes. 6. Put and implement measures to ensure that no transgenic material from the laboratory and the CFT enters the human food or animal feed chain. Contingency measures should also be put in place in case of accidental escape of the trial animals under confinement. 7. All the trial animals and the animal materials must be rendered biologically inactive through autoclaving and or incineration. Strictly adhere to the proposed waste management plan and records must be maintained and availed to bio safety inspectors on request. Any material retained for further analysis shall be counted and accounted for at all times. 8. The applicant to work with DYS to guide them on the data required for vaccine registration. 9. Provide quarterly and annual progress reports to NBA in the prescribed format. The reports should be discussed by and forwarded through the IBC. 10. All staff to be involved in this trial to be trained by DYS and NBA officers on handling transgenic vaccines and overall biosafety and biosecurity matters before the commencement of the trial.	
This approval is not transferrable and is valid for: Five (5) years	
Place: NAIROBI Date: 25th NOVEMBER 2016	Name: WILLY K. TONUI, PhD, RBP, EBS <u>Signature</u> _____ CHIEF EXECUTIVE OFFICER, NATIONAL BIOSAFETY AUTHORITY

Appendix A5.4 Trial Master File Checklist

Table of contents	Documents
Protocol	Current approved version of protocol to funders IACUC Protocol (submitted to ethical board) All previously approved protocols to funders Protocol amendments Protocol deviations
Animal Leasing Agreements (Informed consent)	
Data Collection	Data Collection Forms DCF1 Animal Selection DCF2 Animal Enrolment DCF3 Daily Log of events DCF4 General Health Observations DCF 5 Accounting for IVP DCF6 Accounting for CP DCF 7 Accounting for CV DCF 8 Treatment Administration DCF 10 Clinical Observations/Injection Site Observations/Rectal Temperature DCF 12 Sample Collection and Identification DCF 13 Veterinary Report DCF 14 Temperature Records Freezer DCF 15 Temperature Records Fridge DCF 16 Mortality DCF 17 Necropsy DCF 18 Animal Fate Post-Study DCF19 Shipping Log
Pharmacy	Investigational Product Certificate of Analysis Investigational Product packaging (copy of labels) Import permits Shipping Records
Study Procedures	ILRI Animal unit biosafety manual SOP for Animal Experimentation
Stuy site staff	CV/practice licence for principal investigator and sub-investigators CVs/practice licence for site staff Training Log NBA/DVS Training certificates Duty Delegation Log
Approvals	Initial submission of protocol and any subsequent correspondence Approval letter for protocol Approval letter for IACUC form and all amendments IRB/KEMRI reliance agreement The Pirbright AWERD Approval NBA approval letter
Regulatory	NBA correspondence

Table of contents	Documents
	NBA Approvals
	NBA Quarterly reports
Financial and Legal	Funder documentation
	Lease agreement
	Indemnity of PI/other investigators
	Clinical Trial Agreement
	Subcontractor Agreement
Data Management	Data Management Plan
	- Data acquisition, entry and processing
	- Data queries and data error correction
	- Source documentation requirements
	- Quality review of data SOP
	- Enrolment/Randomisation procedure within protocol
Lab Certification	
Monitoring	Monitoring plan
	Minutes from initiation site visit
	Monitoring reports
	Site Visit Log

Appendix A5.5 Clinical Observation Scoring Guide

Score	General Condition	Depression	Respiratory Distress	Cough	Nasal Discharge	Appetite	Lameness
Absent 0	Clinically normal appearing animal	Bright, alert, responsive Normal behaviour	Normal respiratory effort and rate	No coughing	No nasal discharge	Normal appetite	Weight bearing on all four limbs when moving Moving around pen freely and responsively
Mild 1	Slightly abnormal condition	Reduced responsiveness Otherwise normal behaviour	Slightly increased respiratory effort and rate	Occasional cough	Slight mucus discharge	Slightly decreased appetite	Reduced weight bearing – favouring of limb when moving Moving round pen freely and responsively
Moderate 2	Moderately abnormal condition	Depressed Separates from group Extended resting periods, reluctant to stand, lethargic	Obvious abdominal breathing	Frequent coughing	Mucopurulent discharge	Clearly decreased appetite	Hopping and obvious favouring of limb when moving Extended resting periods Moving only when stimulated

Appendix A7.1 Non-inferiority R Code

```
library("confinterpret", lib.loc="~/Library/R/3.5/library")
NI_Sheep<-read.csv("Sheep_FRNT_NI.csv")
mixmodel = glm(FRNT_d28 ~ TrtGp + Sex + AgeDays_at.day0 +
LWT01, family=binomial, data=NI_Sheep)
summary(mixmodel)
cii<-confint(mixmodel)
ci<-read.csv("ci.csv")
interpret_noninferiority(ci, actual_null = 0, ni_margin =
0.1, groups = c("Smithburn", "ChAdOx1"), beneficial_outcome =
TRUE)
```

Appendix A7.2 Binomial family generalised linear model for seroconversion following vaccination in sheep, goats, cattle and camels

Sheep

Variable	Coefficient	Standard Error	P value
Intercept	4.472340	9.694107	0.645
Treatment Group			
Smithburn	Baseline	-	-
ChAdOx1 RVF	4.629169	0.611978	<0.001
Sex			
Female	Baseline	-	-
Male	0.192849	0.634938	0.761
Age			
Age	-0.018234	0.02972	0.541
Weight			
Weight	-0.009632	0.066746	0.885

Goats

Variable	Coefficient	Standard Error	P value
Intercept	-0.894185	0.769515	0.2452
Treatment Group			
Smithburn	Baseline	-	-
ChAdOx1 RVF	4.047636	0.768361	<0.001
Sex			
Female	Baseline	-	-
Male	0.927242	0.500231	0.0638
Age			
Age	-0.002698	0.002583	0.2962
Weight			
Weight	0.055281	0.062892	0.3794

Cattle

Variable	Coefficient	Standard Error	P value
Baseline	-4.845795	2.013607	0.0161
Treatment Group			
Smithburn	Baseline	-	-
ChAdOx1	4.661829	0.693594	<0.001
Sex			
Female	Baseline	-	-
Male	-0.661220	0.501616	0.1874
Age			
Age	0.006958	0.004249	0.1015

Weight			
Weight	-0.009761	0.010086	0.331

Camels

Variable*	Coefficient	Standard Error	P value
Baseline	-1.948009	1.722386	0.258
Treatment Group			
Smithburn	Baseline	-	-
ChAdOx1 RVF	-0.051651	0.646654	0.936
Sex			
Female	Baseline	-	-
Male	2.871191	0.689021	<0.001
Weight			
Weight	0.000574	0.004318	0.894

*Exact ages were unknown for camels although they met the eligibility criteria of 4 months – 4 years

Appendix A7.3 Cross tabulation for ID Screen ELISA and FRNT₈₀ and Kappa coefficient

Sheep	FRNT ₈₀			
ID Vet ELISA		Positive	Negative	Total
	Positive	11	2	13
	Negative	2	65	67
	Total	13	67	160

Kappa coefficient = 0.82

Sensitivity = 84%

Specificity = 97%

Cattle	FRNT ₈₀			
ID Vet ELISA		Positive	Negative	Total
	Positive	2	0	2
	Negative	1	76	77
	Total	3	76	158

Kappa coefficient = 0.79

Sensitivity = 66%

Specificity = 100%

Goats	FRNT ₈₀			
ID Vet ELISA		Positive	Negative	Total
	Positive	34	4	38
	Negative	2	40	42
	Total	36	44	160

Kappa coefficient = 0.85

Sensitivity = 94%

Specificity = 91%

Camels	FRNT ₈₀			
ID Vet ELISA		Positive	Negative	Total
	Positive	7	5	12
	Negative	5	13	18
	Total	12	18	60

Kappa coefficient = 0.31

Sensitivity = 58%

Specificity = 72%