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Seroprevalence and risk factors for Rift Valley fever in cattle and goats in the Free State and Northern Cape provinces

by

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Submitted in partial fulfilment of the requirements

for the degree Master of Science (Veterinary Epidemiology)

in the Department of Production Animal Studies,

Faculty of Veterinary Science,

University of Pretoria

April 2017

Acknowledgements

First, I will like to thank God Almighty for good health, guidance and blessing me with wonderful people throughout this project.

My sincere gratitude goes to my, father, my royal model, my mentor, and supervisor Prof Peter N Thompson of the Department of Production Animal Studies, for his endless patience, guidance during this research, and for his intellectual criticism, and continues corrections that brought standard to this dissertation.

Special thanks to my co-supervisor, Prof J. T. Paweska of of National Institute for Communicable Disease (NICD) Johannesburg, Special Pathogen Unit (SPU), for his role and assistance in the laboratory work and analysis for this study.

My gratitude goes to the staff of EcoHealth Alliance, most especially Dr M. Rostal and Dr W. Karesh for their invaluable support and coordination of the entire project.

The field and lab work for this project was funded by the Understanding Rift Valley Fever in South Africa Project. The project depicted is sponsored by the U.S. Department of Defense, Defense Threat Reduction Agency. The content of the information does not necessarily reflect the position or the policy of the federal government, and no official endorsement should be inferred.

Special thanks to the staff and management of National Institute for Communicable Disease (NICD) Johannesburg, Special Pathogen Unit (SPU), most especially Dr P. J. van Vuren for their support during the laboratory analysis of the serum samples.

I will also like to thank Dr Claudia Cordel, the field coordinator for facilitating contacts with the State Vets and farmers and for managing all logistics during sample collection.

I am indebted to my colleagues Dr A. Avenant and Mrs V. Msimang for all their support.

My gratitude goes to National Research Foundation (NRF) for their financial support.

I wish to express special thanks to my family, especially my brother Stephen, for all their prayers, love and encouragement.

Summary

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Supervisor: Prof. P.N. Thompson
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Background: Rift Valley fever (RVF) is a mosquito-borne viral zoonosis currently confined to the African continent and the Arabian Peninsula. This study aimed to estimate the prevalence of antibodies to RVF virus (RVFV) in cattle and goats in an area affected by the 2010-2011 outbreaks and to identify factors associated with seropositivity.

Methods: A cross sectional study was conducted during 2015/2016 in a 200 km × 200 km area between Bloemfontein and Kimberley in the Free State and Northern Cape Provinces. Sampling points were selected using random geographic points with probability proportional to density of livestock-owning households. Up to nine cattle and goats were randomly sampled from the farm closest to each selected point. A questionnaire was used to collect information concerning animal, management, and environmental factors. Serum samples were screened for RVFV antibody using IgG indirect ELISA and inhibition ELISA was used for confirmation. Data were analyzed separately for each species by univariable screening followed by multilevel logistic regression models.

Results: A total of 956 cattle and 520 goats were sampled from 232 farms. Overall RVF seroprevalence, adjusted for clustering and sampling weight, was 25.5% (95% CI: 20.8, 30.9%) in cattle and 7.5% (95% CI: 4.3, 12.7%) in goats. Seroprevalence in cattle was highest in Brandfort (50.0%) and Bultfontein (43.5%), and in goats in Jagersfontein (22.2%) and Koffiefontein (11.4%). Seroprevalence in cattle was higher in animals older than 4 years, in dairy cattle, in animals not kraaled at night, on farms that had seasonal pans, and on farms recently (2014-2016) vaccinated against RVF. In goats, seroprevalence was higher in animals

older than 4 years, on private compared to communal farms, and on farms with seasonal pans or permanent rivers or dams, and lower on farms with man-made dams.

Conclusion: Seroprevalence was higher in cattle than in goats, but in both species the level of herd immunity four years after the last reported outbreaks was low. Associations with known environmental risk factors for RVF transmission indicate the possibility that viral circulation has taken place during the inter-epidemic period.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AEC	Animal Ethics Committee
CBB	Carbonate bicarbonate buffer
CC	Conjugate control
CV	Coefficient of variance
DAFF	Department of Agriculture, Forestry, and Fisheries
DIVA	Differentiate infected from vaccinated animals
ELISA	Enzyme-linked immunosorbent assay
HI	Haemagglutination inhibition
ICC	Intra-cluster correlation coefficient
IQC	Internal quality control
NDVI	Normalized difference vegetation index
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
NRF	National Research Foundation
NS	Non-structural
OD	Optical density
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI	Percentage inhibition
PP	Percentage positivity
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SPU	Special Pathogens Unit
VNT	Virus neutralization test

CHAPTER 1 LITERATURE REVIEW

1.1 Introduction

Rift Valley fever (RVF) is a vector-borne viral zoonosis that is currently limited to the African continent, including Madagascar, Comoros and Mayotte, and the Arabian Peninsula. The disease occurs at irregular interval under environmental conditions that favour the reproduction of mosquito vectors (Magona et al. 2013). Outbreaks of RVF may have major societal impacts via human disease, economic losses due to death (predominantly in young animals) and abortion in pregnant sheep, cattle, and goats, as well as banned export of animals and animal products (Archer et al. 2011, Xue et al. 2012). Rift valley fever causes 100% abortion in sheep, 90-100% mortality in lambs, 70% mortality in calves, and animals usually die within 2-3 days. Neonates and exotic breeds are more at risk of RVF infection and mortality. Goats are more resistant than sheep but in some outbreaks similar mortality and abortion rates have been reported. Mortality rate in sheep is 5%-30%, and 5%-10% in cattle. However, in older animals, the mortality rate varies between 5 to 60% (Glancey, Anyamba & Linthicum 2015).

Rift Valley fever virus have been shown to be a potential biological weapon and has high risk of being introduced into Europe and United States through transportation of infected vectors, infected animals and human hosts (Peters, C J. 2002), or intentionally through bioterrorism. The risk factors associated with RVF infection in animals, may include but are not limited to the availability of mosquito vectors, water sources, vaccination status, addition of new animals into the flock, environmental temperature, and rainfall. Change in ecological conditions such as land use, drainage and rainfall, are key players in RFV emergence (Anyamba et al. 2009, Blomstrom et al. 2016, Glancey, Anyamba & Linthicum 2015). Rift Valley fever was first reported in 1951 in South Africa, including but not limited to the Free State; while the last reported outbreak in South Africa was in 2010-2011 in the Free State and the Northern, Eastern and Western Cape provinces (Pienaar & Thompson 2013).

Currently, there is a dearth of information on the seroprevalence of RVF in South Africa. Therefore, the aim of this study was to estimate the seroprevalence of RVF in cattle and goats, to identify factors associated with seropositivity, and to identify if RVF virus (RVFV) has circulated during inter-epidemic period in the study area.

1.2 Aetiology

Rift Valley fever is an arthropod-borne viral zoonosis of the genus *Phlebovirus*, belonging to the family *Bunyaviridae* (Martin et al. 2008, Paweska et al. 2008). Rift Valley fever virus is 90-110 nm in diameter and it is a negative-sense, single-stranded RNA virus characterized by a genome of three segments designated as Large (L), Medium (M) and Small (S). The S segment is ambisense polarity, while the L and M segments are negative-sense (Figure 1) (Murphy et al. 2012, Pepin et al. 2010). The S segment encodes the nucleocapsid protein (N) and non-structural protein NSs. The M segments encodes glycoprotein Gn, Gc and nonstructural protein NSm, while the L-segment encodes the RNA-dependent polymerase (Eliot et al 2013).

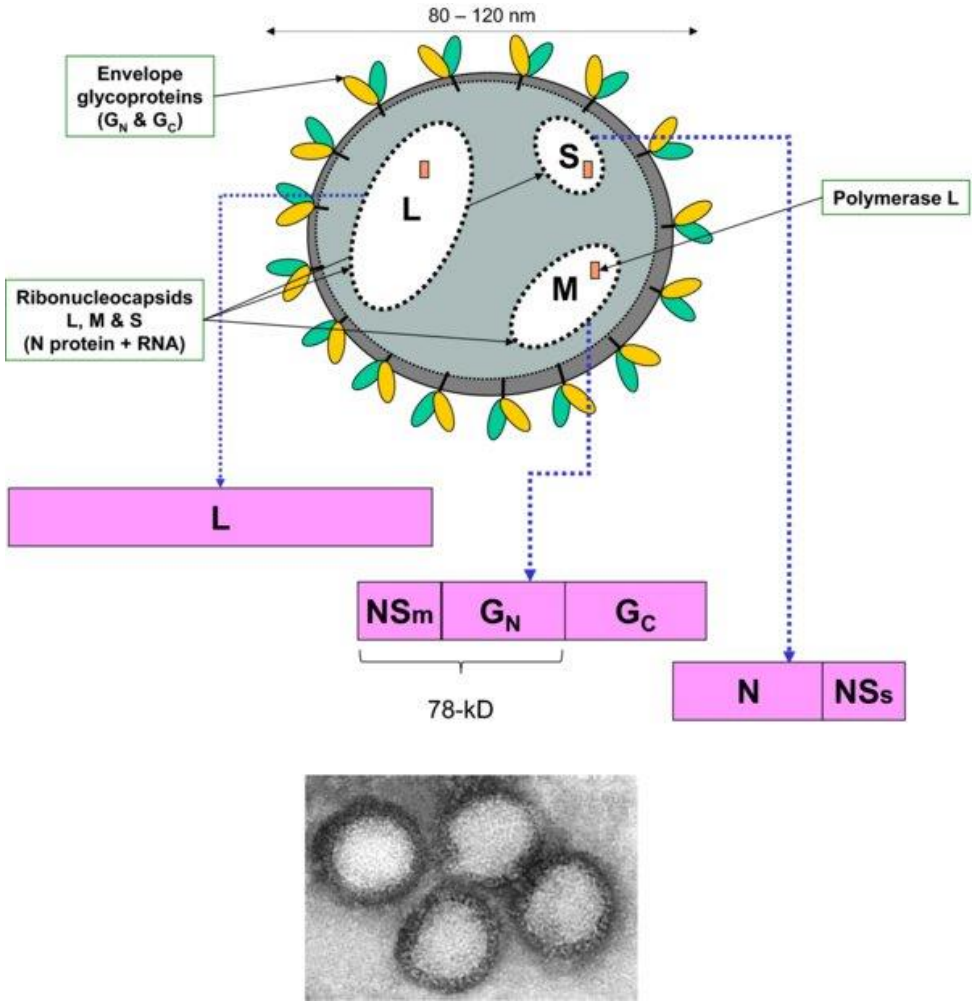

Figure 1. Schematic diagram of Rift Valley fever virus (from Pepin et al. 2010). L is RNA polymerase, N is nucleocapsid, G_N and G_C are glycoproteins, and NS_S/NS_M are nonstructural proteins.

1.3 Epidemiology

The first recorded case of RVF was on a farm near the shores of Lake Naivasha in the Rift Valley of Kenya in 1930 (Daubney, Hudson & Garnham 1931, Kortekaas et al. 2010). Since then, major outbreaks have been reported in Kenya in 1951-53, 1997-1998 and 2006-2007 (de la Fuente et al. 2010, Munyua et al. 2010, Peters & Linthicum 1994, Woods 2002), in Somalia in 1997 (Woods et al. 2002), Mozambique, Zimbabwe and Malawi 1969 (Peters & Linthicum 1994), Sudan 1976, 2007 (Hassan et al. 2011, Peters & Linthicum 1994), Mauritania and Senegal 1987 (Saluzzo et al. 1987, Young, Groocock & Kariuki 1988), and Tanzania (de la Fuente et al. 2010, de la Fuente et al. 2010). These outbreaks had mostly occurred following El Niño events and heavy rainfall causing flooding of dambos (shallow wetlands) except for Mauritania and Senegal where the 1987 outbreaks were linked to the establishment of a dam on the Senegal River.

The first reported case of a RVF outbreak outside sub-Saharan Africa was in Egypt, in 1977-78 (Meegan 1979). In September 2000, there was an outbreak of RVF in Saudi Arabia and Yemen, which was the first recorded case outside Africa, during which about 245 humans and thousands of sheep and goats died (Balkhy & Memish 2003, Blomstrom et al. 2016, Swanepoel & Coetzer 2004). Most recently RVF outbreaks have been reported in Rwanda (Umuhoza et al. 2017), Kenya (Nanyingi et al. 2016), Central African Republic (Nakouné et al. 2016, Nanyingi et al. 2016) and Mauritania (Boushab et al. 2016).

About 20 years after the first recorded case in Kenya, the first reported outbreak in South Africa was in 1950-51 in the western Free State, southern and south-western Transvaal and the northern to western Cape Provinces. It caused an estimated 500,000 abortions in pregnant ewes and about 100,000 deaths in sheep (Abu-Elyazeed et al. 1996, Gear et al. 1951, Meegan 1979, Swanepoel&Coetzer 2004). Mortality in lambs was about 90-95%, in calves 10-15% and in goats <10% (Bird et al. 2009). The second major outbreak in South Africa was in 1974-76, when it was first reported in the district of Bultfontein of the Free State Province and then spread to other provinces (Coetzer 1977). The 1974-76 outbreak was more severe than the 1950 outbreak causing high abortion rate in ewe and high mortality in new born lambs, it was during this period that the first human death was recorded (Pienaar & Thompson 2013). In 2008 RVF re-emerged in South Africa with several outbreaks affecting cattle, goats, sheep, African buffalo and humans in Gauteng, Limpopo, Mpumalanga, and North West Provinces (Archer et al. 2011, Pienaar & Thompson 2013). The outbreak was

diagnosed and confirmed in animals by the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI) and the University of Pretoria, and subsequent outbreaks of RVF were reported in dairy farmers, farm workers, staff of a veterinary hospital, and veterinary students at the veterinary school (Archer et al. 2011, Pienaar & Thompson 2013).

The third major outbreak was in 2010-2011, reportedly affecting 471 and 124 farms respectively, spreading across nearly the whole country and causing extensive livestock deaths (Metras et al 2015). Free State, Northern Cape and, Eastern Cape were severely affected and recorded the first indigenous breeds of livestock and wildlife cases (Pienaar & Thompson 2013).

All the major RVF outbreaks in South Africa were associated with heavy rainfall resulting in increased mosquito populations (Anyamba et al. 2012).

The last major outbreak of RVF in South Africa was in 2010/11 in the interior regions of South Africa, with Free State and eastern Northern Cape provinces as the most severely affected. The epidemic affected mostly sheep, followed by cattle and goats (Pienaar & Thompson 2013). The 2011 outbreak affected mostly Eastern Cape, with some outbreaks in Western and Northern Cape provinces.

Currently there are different types of forecast models used to create RVF risk maps based on information such as sea surface temperatures, outgoing longwave radiation, normalized difference vegetation index (NDVI), and rainfall data gathered from satellites (Anyamba et al. 2009, Anyamba et al. 2010). NDVI is used as an indicator for measuring breeding and increase in vector population (Linthicum et al 1990), and it can be used to correlate climatic condition of different agro-ecological condition in a country to predict an increase in vector population and a possible RVFV outbreak.

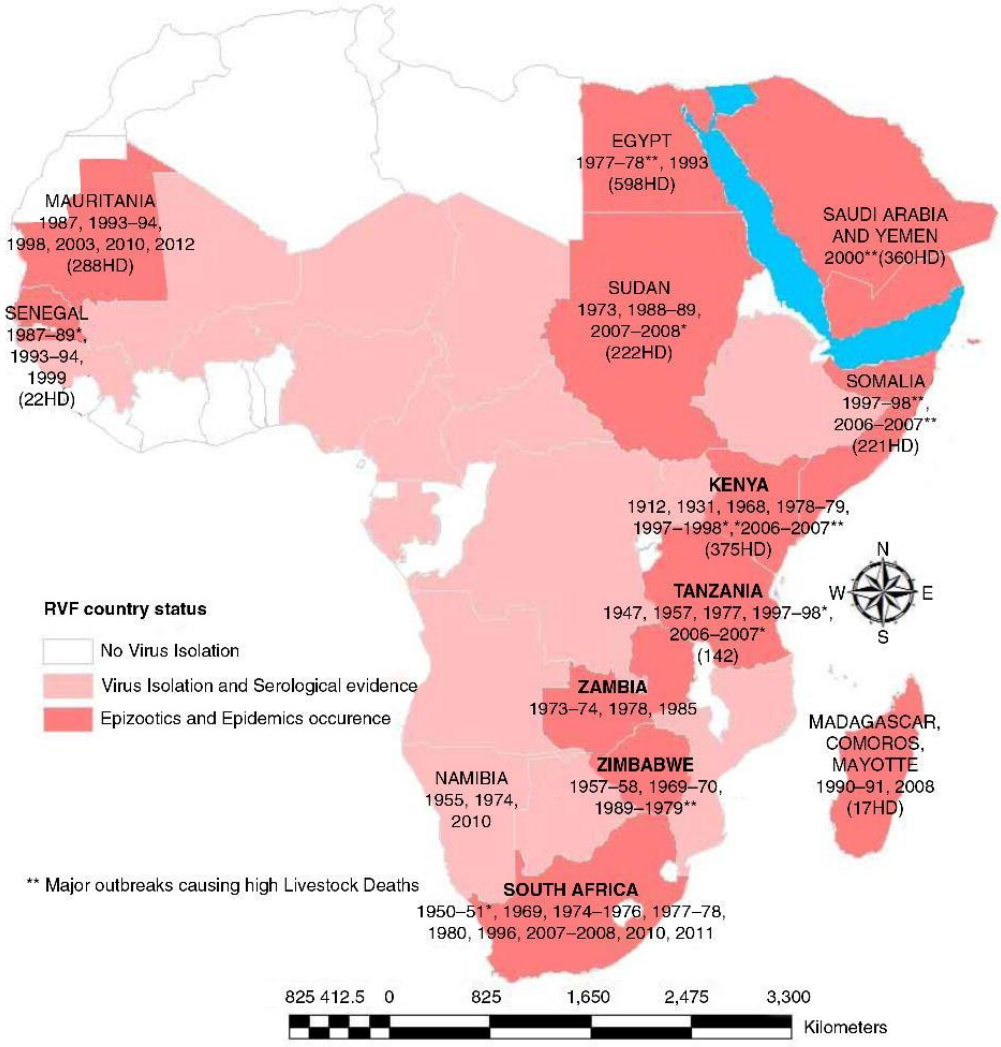

Figure 2. Map illustrating the spatial and temporal distribution of RVF (1912-2012) (Nanyingi et al. 2015).

In Southern Africa, RVF outbreaks occur at long intervals and the outbreak usually starts in mid to late summer following high rainfall and flooding that fill dambos (shallow wetlands), which provide suitable mosquito breeding and egg hatching that subsequently infect humans and livestock with RVF during the process of feeding. (Linthicum et al 1984, Spickler 2006, Woods et al. 2002, Swanepoel & Coetzer 2004).

Human activities such as dam building and flooding due to irrigation may also facilitate the breeding of mosquitoes (Pienaar & Thompson 2013). However, in some areas, where mosquito population is nearly zero transmission of RVF does not occur during dry season (Linthicum et al. 1985).

The mechanism of RVFV survival during inter-epidemic period and before each season remains unclear. Different species of mosquitoes have been demonstrated to transmit RVFV worldwide including *Culex* spp. in areas with permanent water; however, the genus *Aedes* may be important for the virus maintenance during the inter-epidemic period (Pepin et al. 2010). Some *Aedes* spp. lay eggs in wetland habitat, and the eggs can survive in the soil for a long period until they are flooded after high rainfall, which facilitate the hatching of the infected eggs and development into immature stage (Tantely et al 2015). In Kenya and South Africa, wildlife species such as African buffalo (*Syncerus caffer*), may play an important role in RVFV maintenance during an inter-epidemic period (Evans et al 2008, Olive et al 2012, LaBeaud et al 2011). However, there has been no evidence of RVF antibodies in giraffes (*Giraffa camelopardalis*), lions (*Panthera leo*) and wild equines (*Equus burchelli*) (Evans et al. 2008).

In Western and Central Africa, due to the abundance of rainfall, the patterns of RVFV transmission differ from that of Southern Africa. In Western Africa, RVFV transmission follows a more continuous form and most likely depends on high rainfall that favors the breeding of mosquitoes and the presence of high population of susceptible animals (Bird et al. 2009). In West and Central Africa, RFV manifests less as compared to the disease in Southern Africa (Adeyeye, Ekong & Pilau 2011, Nakouné et al. 2016).

There has been no published study of seroprevalence of RVFV in domestic ruminants in South Africa; however, LaBeaud et al. 2011 conducted a study on RVFV infection among African buffalo (*Syncerus caffer*) in Kruger National Park and reported a seroprevalence of 21%. A seroprevalence of 33.5% and 30.6% in goats and cattle respectively was reported in Rebublic of Comoros (Roger et al. 2011), 3% in goats was reported in Kenya (Rostal et al. 2010), 28% in cattle was reported in Madagascar (Chevalier et al. 2011), 21.3% and 11.6% in goats was reported in 2007 and 2010 in Zambezia Province, Mozambique (Fafetine et al. 2013, Fafetine et al. 2007). A more recent study reported RVFV seroprevalence of 25.1% in goats in Zambezia province of Mozambique (Blomstrom et al. 2016), which was more than the 2007 and 2010 reported seroprevalence from the same province. Seroprevalences of 25.8% and 33.3% in goats and cattle respectively were recently reported in Garrisa, Kenya (Nanyingi et al. 2016), while a seroprevalence of 16.8% in cattle was reported in Rwanda (Umuhoza et al. 2017).

1.4 Transmission

RVF is transmitted by mosquitoes, predominantly of the genera *Aedes* spp. and *Culex* spp. (Metras et al 2015, Le Coupanec et al 2013). Trans-ovarial transmission have been reported where the virus survives in eggs of *Aedes* mosquitoes in dambos or wetland environment for a long period of time until the next season. It might also be through low-level transmission between mosquitoes and animals (Manore & Beechler 2015, Pienaar & Thompson 2013).

Another known means of transmission is by direct contact with infected blood or foetal fluids (Bird et al. 2009, Chevalier et al. 2005, Taylor et al. 2016).

It has been reported that animals can be infected during mass vaccination with an automatic syringe and needle especially when an animal's viremic titre is high (Swanepoel & Coetzer 2004). Rift valley fever virus is rarely transmitted to humans by mosquito bite (Fischer et al. 2013). The most common ways humans get infected is by contact with infected blood, body fluids or tissue of infected animals, which usually occur when conducting surgery, post-mortem, and disposal of carcasses or foetuses of infected animals and, rarely, by consumption of unpasteurized milk of infected animals. Occupational groups such as agricultural workers, abattoir workers, farmers, herders, and veterinarians are at higher risk of being infected with the virus (Bird et al. 2009, Ellenbecker et al. 2012).

Transmission of RVFV to unaffected areas usually occurs when infected animals travel to uninfected locations where competent vectors exist. Such vectors (uninfected mosquitoes) can contract the virus from infected animals through blood meal and transmit it to uninfected but susceptible animals, including humans, with possible resultant outbreaks (Mondo, Diallo & Digoutte 1995). Another possible route by which RVFV is introduced into a new location might be windblown infected mosquitoes, and infected passengers on commercial airlines (Hoogstraal et al. 1979). Aerosol transmission during laboratory procedures has been demonstrated (Ikegami 2012).

1.5 Clinical signs

Abortion storms characterize RVF, with high mortality seen in neonatal animals and 10 to 20% mortality among adult livestock (Bird et al. 2009). Abortion is the main clinical manifestation of RVF in pregnant livestock, with up to 100% in pregnant ewes and <10% in pregnant cows reported. Mortality of up to 100% in lambs that are less than 5 - 6 days old can be recorded. However, calves are less susceptible (mortality of 10% - 70%) and their survival

rate is higher compared to kids and lambs. Clinical signs include anorexia, high fever, lymphadenitis, nasal discharges, salivation, bloody diarrhoea and lacrimation in mature animals. Abdominal colic, jaundice, and temporary decrease in milk production in lactating cows are apparent. Epidemic periods usually last for 8-16 weeks (Anyamba et al. 2010, Coetzer 1977, Daubney, Hudson & Garnham 1931, Erasmus & Coetzer 1981).

Very little information on the pathogenesis of the disease in goats has been documented. Although goats are susceptible, they are more resistant than sheep (Chevalier 2013, Nabeth et al. 2001). However, previous studies (Ksiazek et al. 1989, Nabeth et al. 2001) showed that in West Africa, about 2-10% of goats are seropositive for anti-RVFV antibody during interepizootic period. Meanwhile during subsequent epizootics, this amount has risen to more than 70% and high mortalities have been reported in kids (Bird et al. 2009).

Clinical signs in human are associated with fever (38.8-40°C), headache, muscular pain, weakness, and nausea, although asymptomatic cases are most common. A severe disease is seen in 1-2% of affected individuals, with acute hepatic necrosis, hepatitis, encephalitis, retinitis. Permanent blindness among hospitalized individuals with a case fatality proportion of 10 to 20% is common (Bouloy & Weber 2010, Jäckel et al. 2013, Madani et al. 2003)

1.6 Pathology

Post mortem lesions in all species are characterized by hepatic lesions that are severe and extensive in young animals and aborted foetuses; the liver is large, dark tan coloured, soft and friable, with irregular patches of congestion. Hepatic lesions are less severe and more localised in calves and adult animals, and may consist of numerous pinpoint reddish to greyish-white necrotic foci (Spickler 2006). Visceral haemorrhages often accompanied by oedematous gallbladder, and abomasal and intestinal haemorrhages may occur (Coetzer 1977, Swanepoel & Coetzer 2004).

1.7 Diagnosis

Abortion up to 80-100%, high neonatal mortality, and high mortality of up to 10-30% in adult sheep, 5-10% in cattle and <10% in goats, are indicative of RFV disease (Swanepoel & Coetzer 2004).

The methods of diagnosis of RVF include the detection of virus-related antigen and antibody from clinical specimens. Serological assays such as enzyme-linked immunosorbent assay

(ELISA), indirect immunofluorescence, hemagglutination inhibition (HI), and virus neutralization tests (VNT) are often employed in the diagnosis of RVF from clinical specimens.

Reverse transcriptase Polymerase Chain Reaction (RT-PCR) is used to detect viral RNA and it has been reported to be essential and novel for the detection of RVFV (Bird et al. 2009, Garcia et al 2001).

1.7.1 Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a serological immunoassay used to confirm the presence of IgM antibodies which appears immediately after the body has been exposed to an infection as an early transient response. It also confirms the presence of IgG antibodies which indicates previous exposure to an infection, which persist for several years.

Enzyme-linked immunosorbent assay generally has high sensitivity and specificity; it is cost effective and can be used to assay a large number of serum samples within a short period (Paweska et al. 2003). ELISA has been used to detect virus-related antigen antibody in humans and arthropods (Meegan 1979).

1.7.2 Indirect ELISA

This involves the coating of antigen (RVFV recN) and antibody (present in test sera) on a micro titre well that react and bind to the micro titre plate. The unbounded free antibodies are washed away subsequently in every stage, while the bound antigen antibody is detected by adding protein G HRPO conjugate enzyme. ABTS substrate/chromogen is added to yield color. The reaction is quantified by using micro titter plate reader at 405nm (Crowther 2000, Van Vuren 2012).

Indirect ELISA is used as a proxy for VNT for RVFV serology (Paweska et al. 2008) Compared to VNT results, indirect ELISA had a sensitivity of 94.56% and a specificity of 95.57% (Jäckel et al. 2013).

Also, Paweska et al. 2008 reported a sensitivity of 98.7% and specificity of 99.3% from a study conducted on sera of buffalos using nucleoprotein ELISA. Furthermore, Fafetine et al. 2007 reported sensitivity of 99.4% and100% and a specificity of 98.3% and 100% from cattle and small ruminants respectively. Compared to Sandwich ELISA, IgG indirect ELISA

results, in sheep and goats, yield sensitivity of 88.04% and 98.46% and a specificity of 99.81% and 100% respectively (Fafetine et al. 2013). Compare to VN and HI tests, indirect ELISA yields a slightly lower sensitivity but the difference is insignificant in early stage detection of experimentally induced immunological responses when used in population based survey (Paweska et al. 2003).

Indirect ELISA has high sensitivity and antibodies bind to more than one antigen. It is cost effective and flexible. However, cross reaction might occur with secondary antibody yielding non-specific reaction.

1.7.3 Inhibition ELISA

This involves the measurement of either antigen or antibody concentration in a sample. It detects IgG and IgM antibodies against RVFV in humans, domestic and wild ruminants (Paweska et al. 2005)

Anti-RVF antibody is incubated with RVF antigen, the antigen antibody complex is then added in 96 well plates, that was pre-coated with the same antigen. The unbounded antibody is removed by washing the plates. Anti-rabbit IgG HPRO conjugate binds to rabbit anti-virus rN antibody, then ABTS substrate/chromogen is added and causes green coloration of the negative well. The reaction is quantified by using micro titter plate reader at 405 nm (Paweska et al. 2005).

Inhibition ELISA and VNT yielded a similar immunological result to experimental vaccination with live-attenuated and inactivated RVFV. Compared to VNT, Inhibition ELISA is more sensitive for the detection of earliest immunological response in the sera of experimentally vaccinated human and animal (Paweska et al. 2005). In determining recent infection, Paweska et al. have reported sensitivity of 99.45% in humans and specificity of 100% in sheep, buffalo, and camels (Paweska et al. 2005).

1.7.4 Haemagglutination inhibition

Although haemagglutination inhibition (HI) is sensitive, it is labour-intensive and cross reactions with other species of phleboviruses genus may occur; therefore, when using HI, sera from previously infected animals with phleboviruses other than RVF might be positive (Scott et al. 1963).

1.7.5 Virus neutralisation tests

The shortcoming of VNT is that it is relatively expensive. It can only be carried out under biosafety level three (BSL3) lab conditions (Pepin et al. 2010). In addition, the results take ≤ 7 days to be ready which are unsuitable in epidemic situations.

VN and HI tests are classical diagnostic methods for the detection of antibodies to RVFV. The disadvantages from these techniques include health risk to laboratory workers, limitations for their use outside RVF endemic areas and inability to differentiate between different classes of immunoglobulins and lack of standardization and inappropriateness for screening large numbers of sera (Fafetine et al. 2007, Jäckel 2015, Paweska et al. 2003).

1.8 Prevention and control

The movement of livestock and livestock products from infected to uninfected area has been shown to propagate the spread of the virus (House, Turell & Mebus 1992). Therefore, restriction of movement of animal and animal products may be effective in slowing the spread of the virus from endemic to none endemic areas (Centers for Disease Control and Prevention 2012). Active veterinary surveillance program to detect and report new suspected cases is crucial to provide primary warning to veterinary and public health authorities.

1.8.1 Vaccination

Outbreaks of RVF in animals can be prevented by a sustainable animal vaccination programme. In South Africa, RVF immunization is recommended before the beginning of the rainy season, before vector activity is high and outbreaks are more likely. Because of high economic impact of RVF outbreaks, routine immunization of young animals from six months old is recommended. Three types of RVF vaccine have been developed by Onderstepoort Biological Products for animal use in South Africa. They are:

1. The live attenuated Smithburn RVF Vaccine, derived from Smithburn's neutrotropic strain, is used in all breeds of cattle, sheep, and goats. However, it may cause abortion and foetal abnormalities in pregnant animals but elicit lifelong immunity (Barnard 1979, Gerdes 2002, Smithburn 1949). It is commonly used in East and southern African countries, as well as in the Middle East. To date, the vaccine is still widely available for use in the endemic region. Smithburn RVF vaccine is not recommended for use in

countries where RVFV has not been reported because of its potential to revert to virulence (Swanepoel & Coetzer 2004).

2. The RVF Clone 13 vaccine is a live weakened strain that is characterized by a several deletions of gene encoding for the virulent factor known as non-structural (NS) protein (Muller et al. 1995). It has no side effects in livestock, and this was recently introduced for use in cattle and sheep as single dose in South Africa. New-born animals from susceptible ewes or cows can be immunized within 10 days with RVF Clone 13 vaccine (von Teichman et al. 2011)
3. Inactivated RVFV is a formalin-inactivated vaccine that results from a field isolate of RVFV modified to growth in cell culture (Barnard & Botha 1977). The shortcoming of this vaccine is that it requires a booster dose twice after the initial vaccination, before subsequent annual booster. The advantage of vaccine is that it can be used during an epidemic and in pregnant animals, unlike the attenuated Smithburn vaccine which is inappropriate for use in this group.

To prevent RVF before an outbreak, a very effective vaccination program must be implemented and carried out. Once an outbreak has occurred it is advised that animals should not be vaccinated, because this might intensify the outbreak (Centers for Disease Control and Prevention 2012).

CHAPTER 2

2.1 Aims of the study

To date, there is no known, published seroprevalence study of RVF in livestock in South Africa. This study will therefore serve as a baseline survey, it will provide the seroprevalence of RVF in cattle and goats in the study area. It will also present seroprevalence variations by districts, age, and species. Risk factors associated with seropositivity will be identified, and possible evidence of inter-epidemic circulation of RVFV in cattle and goats in the study area will be assessed.

2.2 Objectives

1. To estimate prevalence IgG antibodies to RVF in cattle and goats in a study area in the western Free State and adjacent Northern Cape provinces of South Africa.
2. To determine if seroprevalence varies between geographic regions and age groups of cattle and goats.
3. To identify factors associated with seropositivity by correlating serological results with potential risk factor information.
4. To determine whether natural circulation of RVFV had occurred in the study population since the last outbreak in 2011.

CHAPTER 3: MATERIALS AND METHODS

This cross-sectional study was part of a larger project, where several monitoring sites were established and environmental and vector data were collected. Whereas this study focused on serological surveillance in cattle and goats, other collaborators focused on humans, sheep, and wildlife. Information on risk factors for this study were collected at all established sites. All the studies were carried out concurrently.

3.1 Ethical Considerations

Prior to the commencement of this study, the University of Pretoria Animal Ethics Committee (AEC) approved the protocol for this study (Project number V090-15) (Appendix C). In addition, the Department of Agriculture Forestry, and Fisheries (DAFF) approved this study in terms of Section 20 of the Animal Diseases Act of 1984 (Appendix D).

3.2 Consent Form

Prior to the commencement of sampling on each farm, farm owners were given a consent form explaining in detail the background and purpose of the research, detailed procedures to be performed, benefits and risks of participation in the study. The farmers were asked to sign the informed consent form if they consented to participate in the study (Appendix B).

3.3 Confidentiality

All information gathered or obtained during this study were held in strict confidentiality. The data generated during this study were handled by authorized individuals only. The questionnaire (Appendix A) was designed for use on a tablet and translated into local languages, was administered to farm owners using electronic tablets by trained individuals and the responses were uploaded into the project database via the internet. The data were later downloaded into an Excel file to provide additional farm information on environmental and management variables for analysis by the researchers only.

3.4 Model system and study design

3.4.1 Study area

A cross-sectional study was conducted during 2015-2016 within a ~40,000 km^2 region between Bloemfontein and Kimberley (Fig. 1). Bloemfontein is the capital city of Free State Province, situated on dry savannah at 29°06'S 26°13'E, at an altitude of 1,395 m above sea level. Based on the 2011 census, the city is home to 464,591 residents (City population, 2015). Kimberley is the capital of Northern Cape Province, located approximately 110 km east of the confluence of the Vaal and Orange Rivers. Situated at 28°44'S 24°46'E at an altitude of 1,224 m above sea level, it contains about 225,160 residents. This region was selected for this study because it was where most of the outbreaks in 2010-2011 were reported.

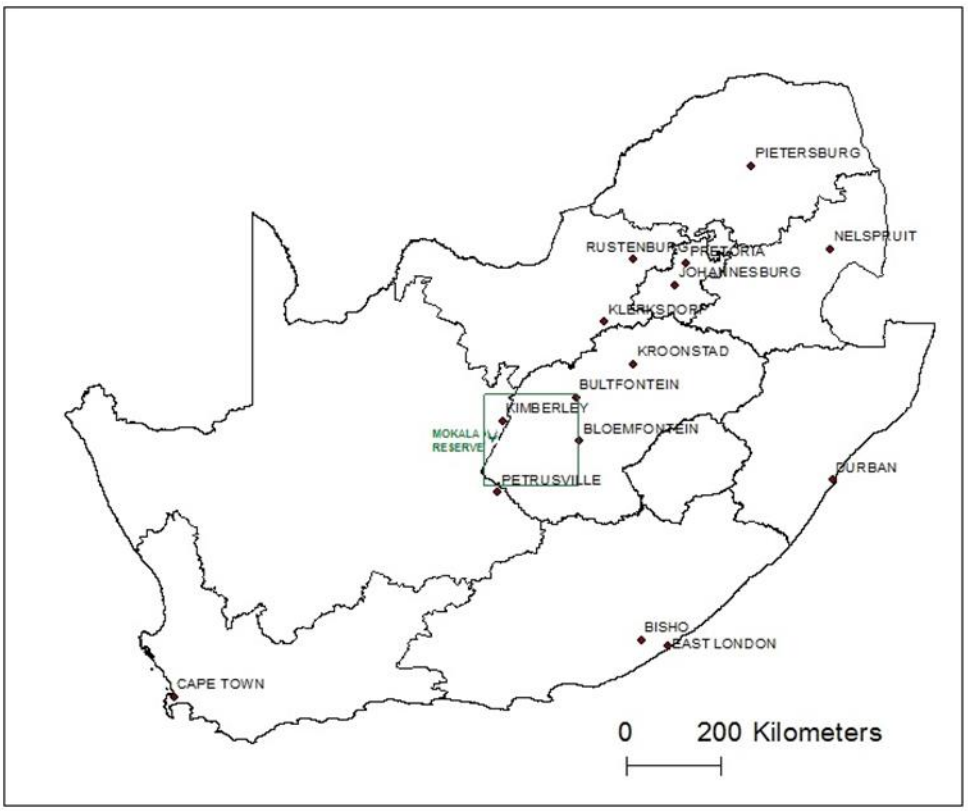

Figure 3. Map of the study area

Study animals were kept under normal farming conditions. All blood samples were collected on the farm without interference in the management system and animal biodata and other demographic information were collected.

3.4.2 Survey design

About 500 geographic points were randomly generated, with selection probability proportional to the density of livestock-owning households obtained from the results of the 2011 National Census (K. Parry, Statistics South Africa, 2014, *pers. comm.*). The points were randomly sorted and were plotted on a Google Earth map of the study area, making it easy to locate farms.

Geographic coordinates of farms were obtained from points plotted on a Google Earth map, making it easy to travel to the farms nearest to the selected point. Farmers' phone numbers were obtained from the farmers' union office or police stations at the districts. Farmers were called and briefed about the project to book possible appointment for sampling. In situations where farmers phone number could not be obtained, we drove to the farm and informed the farmer about the project. When a farmer declined to participate in the survey or there were no animals on the farm, the next nearest farm with animals was used.

The population of all the cattle and goats in the study area was considered as the target population, including animals on commercial, smallholder and communal farms. Random geographic points were used to select farms since no sampling frame of all farms in the study area existed. Cattle and goats were randomly selected for sampling from the farm closest to each selected sampling point. This was therefore a multistage random sampling survey design.

To investigate the prevalence in different age categories on the farms, animals were divided into three age categories: 6 months to 2 years of age, 2-4 years of age (born since the last outbreak) and >4 years old (those alive during the last outbreak in 2011). Where possible, animals were stratified by age category, with three animals of each species selected within each category. In many cases a combination of haphazard and convenience sampling was used, with farm workers selecting animals from each age category. If the required number of animals in each age group was not available, additional animals were selected from the other age groups. A maximum of nine cattle and nine goats was sampled per farm. In cases where the number of cattle or goats on the selected farm was less than nine, all the available cattle or goats were sampled.

The next point on the list was then selected, sampling a maximum of nine cattle and goats per farm until the required total sample size for each species was fulfilled. Informed, written

consent (Appendix B) was obtained from all owner of the animals used in this study. However, if the farm owners did not consent to their animals being used, then the next closest farm to the selected point was selected.

3.4.3 Sample size calculation

Sample size was calculated using the formula:

$$n = \frac{Z^2 P_{exp}\, Q}{L^2}$$

Where n is the sample size, P_{exp} is the expected prevalence; L is the precision of the estimate (also called "the allowable error" or margin of error) which is equal to half the width of the confidence interval; Q = 1-$P_{exp.}$ and Z is the $(1-\frac{\alpha}{2})$ percentile of a standard normal distribution (Dohoo, et al., 2003); for α = 0.05, Z = 1.96.

Due to lack of recent data on seroprevalence of RVF in cattle and goats in Southern Africa, based on reported seroprevalence elsewhere in Africa, it was decided to use an expected prevalence of 15% (Fontenille et al. 1998, Rostal et al. 2010, LaBeaud et al. 2007) for the sample size calculation, giving a required sample size of 196 for each species.

Because two-stage sampling was done, with individual samples clustered within farms, the standard error of the prevalence estimate was inflated compared to that which was calculated assuming simple random sampling (Bennett et al. 1991). Therefore, it became necessary to increase the sample size, to compensate for the reduction in precision.

Then, the design effect was calculated by manipulating the formula (Otte & Gumm 1997):

$$\rho = \frac{D-1}{n-1}$$

Where ρ is the intra-cluster correlation coefficient (ICC), D = Design effect and n = Average cluster size. The expected design effect can be calculated by rearranging the above equation to:

$$D = 1 + \rho(n-1)$$

A value of ρ = 0.2 was assumed, as reported from previous studies of analysing surveillance data of various diseases ρ rarely exceeds 0.20, although the ICC varies among different

infectious agents (Otte & Gumm 1997). A value of nine animals within cluster of farms was decided to be sampled for convenience per species, making three animals for each age category. Using ρ = 0.2 and n = 9, a design effect D = 2.6 was calculated. A total sample size of 510 cattle and goats each was therefore calculated using the formula:

$$N_{adj} = N_{unadj} \times D$$

Where N_{adj} is the adjusted sample size, N_{unadj}= Unadjusted sample size and D = Design effect.

A total of 956 cattle and 520 goats were sampled instead of the required 510 cattle and goats each. This was due to the fact that sufficient farms had to be sampled to obtain the required numbers of all the species (cattle and goats). Also, due to availability of funds to test more animals, it was decided that to sample more than nine animals on farms. At the end, we decided to sample all goats on farms to achieve the desired sample size.

3.5 Questionnaire Design

The questionnaire (Appendix A) was pre-tested on 19 farms before the commencement of the project. It was designed as closed-ended and translated into two local languages (Sesotho and Afrikaans) and was administered to farm owners or managers using electronic tablets by trained individuals; however, repeatability between these individuals was not assessed. The responses were uploaded into the project database via the internet for storage. The data were later downloaded into an Excel file to provide additional farm information on environmental and management variables for analysis. Data were transferred into Stata 14 for analysis (StataCorp, College Station, TX, U.S.A.).

3.6 Blood Sampling

Animals were identified by age (determined by dentition and/or farm records), sex, species, breed, location of sample collection, farm name/address, sample type and GPS coordinates. Blood collection tube was properly labeled with animal ID and collection date.

After the animals were randomly selected, cattle were placed in a crush and a blood sample was then drawn via the tail vein located in the midline about 10 cm from the base of the tail. In a situation where blood sample had to be collected via the jugular vein, a halter was placed over the head and the rope round the nose of the cattle; this tilted the head to the side of the crush and exposed the jugular vein. Goats were restrained in a crush or pen against the fence,

and were restrained between the legs and held by the jaw turning their head at a 30° angle to the side to allow easy access to the jugular vein.

Using acceptable venipuncture technique, a blood sample was collected observing universal precautions; 8.5-17 ml of blood was collected using a 20-gauge needle in goats and an 18-gauge needle in cattle. An 8.5 ml Vacutainer® tube with clot-activator and gel for serum separation (gold stopper) was used and allowed a minimum of 15 minutes for the blood to clot, after which the sample was centrifuged in the field using a portable centrifuge machine (Beckman Coulter Allegra™X-22 at 1000 revolution/min) to separate the serum from the clot and packaged for transportation to National Institute for Communicable Diseases (NICD) Special Pathogen Unit (SPU) Johannesburg.

The package was placed in a Thermocool box or small cooler with ice packs to maintain proper cold-chain system during transit. The boxes were sealed securely and clearly labelled as “Biohazard material” and send to the laboratory as soon as possible where it was stored at -20°C until analysed. The samples were aliquoted in duplicate in a volume of about 1-2 ml transferred into properly labelled, leak-proof unbreakable plastic container with screw cap, with animal ID and serum collection date. The serum samples were stored in a walk through cold room at -20°C.

3.7 Laboratory analysis

Serum samples were screened using an indirect ELISA (i-ELISA) for the detection of IgG against RVFV and positive results from this were confirmed using inhibition ELISA. The procedures were performed using validated protocols at the NICD Special Pathogens Unit (SPU) of the National Health Laboratory Service (NHLS).

3.7.1 Indirect ELISA

The procedure for validation of the indirect ELISA based on RVFV recombinant antigen (RVFV recN) was conducted as previously described (Paweska, van Vuren & Swanepoel 2007). The Nunc MaxiSorp^{R} high protein-binding capacity 96 well ELISA plates were used. The plates were coated with 6 µl of RVFV recN antigen to 12 ml carbonate bicarbonate buffer (CBB) (1:2000), then 100 µl/well of the CBB was added to the plate, covered, and refrigerated at 4°C overnight. It was then washed 3 times with phosphate buffered saline (PBS) 0.1% tween-20 using ELx405 Auto Microplate washer (Bio-Tek Instrument, INC ELISA plate washer). A volume of 200 µl/well blocking buffer were added to the plate, covered, and incubate at 37°C for 1 hour in a moist chamber and this was then washed 3 times with PBS, and 100 µl of test and control sera (C++, C-) diluted 1:400 in PBS were added in pairs into wells and were incubated at 37°C for 1 hour in a moist chamber. The Conjugate control wells (CC) consist of only diluent buffer with no serum added (Figure 4. Plates were then washed three times with PBS 0.1% tween20, then 100 µl/well of protein G HRPO conjugate diluted 1:2 000 in diluent buffer were added and incubate at 37°C for 1 hour in a moist chamber, after which the plates were washed 3 times with PBS 0.1% tween-20. A volume of 100 µl of ABTS per well was then added. Plates were incubated for 30 minutes at room temperature (22-25°C) in the dark and then 100 µl of 1x concentrated SDS (stop solution) was added to stop the reactions. Optical density was read at 405 nm using an Elx800 Universal Microplate Reader (Bio-Tek Instruments, INC).

	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	C++	1	2	3	4	5	6	7	8	9	10
B	C++	C++	1	2	3	4	5	6	7	8	9	10
C	C-	C-	11	12	13	14	15	16	17	18	19	20
D	CC	CC	11	12	13	14	15	16	17	18	19	20
E	21	22	23	24	25	26	27	28	29	30	31	32
F	21	22	23	24	25	26	27	28	29	30	31	32
G	33	34	35	36	37	38	39	40	41	42	43	44
H	33	34	35	36	37	38	39	40	41	42	43	44

Figure 4. Indirect ELISA plate template

**Plate layout: C++ = Positive control serum; C- = Negative control serum; CC = Conjugate control; 1-40 = Test sera*

The degree and intensity of colour formed is relative to the amount of anti-RVFV IgG antibody that has bound to RVFV recN and is accessible to react with the detection system.

There are three levels of microplate acceptance criteria. The result on a test plate is recognized as valid if it fulfils the first level of internal quality control (IQC) by at least three of the optical density (OD) values recorded for C++ fall within the range 0.9 (lower control limit) to 2.0 (upper control limit). The result is not recognized if two or more of the replicates of C++ fall outside IQC limits; it therefore needs to be repeated. The plates are recognized if at least two intermediate OD values of C++ are used for the calculation of the mean OD value of C++. This value is then used in the succeeding calculations of percentage positivity (PP) of C++, C- and test sera as follows:

$$\text{PP} = \frac{Mean\ OD\ serum\ (\text{C++,or C}-\text{,or Test serum})}{Mean\ OD\ \text{C++}} \times 100$$

The second level is recognized only when the results attained on a test plate fulfil the second level of IQC, if the coefficient of variance (CV) for PP values of two reproduced of C++ obtained from intermediate OD value is less than 10%. The CV is calculated as follows:

$$CV = \frac{\text{Standard deviation of replicates}}{Mean} \times 100$$

Threshold PP values of bovine sera must be ≥30 PP, and sheep and goat sera must be ≥25 PP before they are considered positive and any values less than this are classified as negative.

3.7.2 Inhibition ELISA

The inhibition ELISA was carried out as previously described (Paweska et al. 2005). The Nunc MaxiSorp^{R} high protein-binding capacity 96 well ELISA plates were used. This plate requires 12000 µl (12 ml) of 1% PBS for 1:500 dilutions. Therefore, 12 ml of 1% PBS will require 24 µl of polyclonal sheep anti-RVFV capture antibody (12000 µl + 24 µl sheep anti-RVF capture antibody). A volume of 100 µl of the mixture was added per well of the ELISA plate and incubated over night at 4°C in a moist chamber. After incubation, plates were washed three times with 0.1% Tween-20 in PBS, this washing procedure was done on each subsequent stage of the assay. Blocking was done by adding 200 µl per well of 10% skimmed milk in PBS (blocking buffer) and was incubated for 1 hour in a moist chamber at 37°C.

During the blocking stage, 500 µl of RVF antigen (strong positive) Ag++ was mixed with 5 ml of 2% skimmed milk. A volume of 189 µl of virus antigen (500 µl Ag++ plus 5 ml 2% skim milk) were added to rows of the top half of the plate (rows A3-A12, C3-C12, B1, C1, and D1) and 189 µl of negative virus antigen (500µl Ag- plus 5 ml 2% skim milk) were added to the bottom half of the plate (rows E3-E12, G3-G12, F1, H1, and H1) and 21 µl/well of positive and negative undiluted test sera was added to rows A3-A12, E3-E12 and C2-C12, G3-G12 consecutively in corresponding order, while 21 µl/well of positive sheep anti RVF IgG (C++) plus 189 µl of 2% skim milk were added to well B1 and F1. A volume of 21 µl/well of negative sheep RVF control sera (C-) plus 189 µl of 2% skim milk were added into wells C1, D1, G1 and H1. But for the CC wells, 200 µl of undiluted 2% skimmed milk were added.

A volume of 100 µl/well of the mixture was transferred in to the plate in a corresponding order. Starting from row A3-A12 goes to A3-A12 and B3-B12. C3-C12 goes to C3-C12 and D3-D12 since the volume is for duplicate testing. A volume 100µl of test and control sera/virus antigen mixture was added to rows A-D: 1-12, while 100 µl of control antigen mixture to rows E-H: 1-12 (see figure 4), and incubated for 1 hour in a moist chamber at 37°C after which plates were then washed.

A volume of 100 µl/well of rabbit anti-virus rN diluted 1:2000 in diluent buffer were added and incubate for 1 hour in a moist chamber at 37°C, after which the plates was washed. A volume of 100 µl/well of anti-rabbit IgG HRPO-conjugate diluted 1:6000 in diluent buffer were added and incubate for 1 hour in moist chamber at 37°C and the plates were washed 6 times, then 100 µl of ABTS/well was added and the plates were left for 30 minutes at room temperature (22-25°C) in dark, after which 100 µl of 1 x concentrated SDS stop at 405 nm.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CC	CC	1	5	9	13	17	21	25	29	33	37
B	C++	C++	2	6	10	14	18	22	26	30	34	38
C	C-	C-	3	7	11	15	19	23	27	31	35	39
D	C-	C-	4	8	12	16	20	24	28	32	36	40
E	CC	CC	1	5	9	13	17	21	25	29	33	37
F	C++	C++	2	6	10	14	18	22	26	30	34	38
G	C-	C-	3	7	11	15	19	23	27	31	35	39
H	C-	C-	4	8	12	16	20	24	28	32	36	40

Figure 5. Inhibition ELISA plate template

A specific activity for each serum (net OD) was calculated by subtracting the non-specific OD in wells with virus antigen. The mean OD readings for replicate test were converted to a percentage inhibition (PI) value using the equation below:

$$\text{PI value} = \frac{100 - \text{mean net OD of test sample}}{Mean\ net\ OD\ of\ negative\ control} \times 100$$

Sera were tested in duplicates using ELISA technique, as shown in Figure 4.

During substrate incubation, the net optical density of negative serum control (OD C-) reaches the internal quality control (IQC) limits after 30 min. Before adding SDS to stop

coloration, there plates were pre-read and incubation of the substrate was extended for additional 10-15 min.

The cut off values for upper and lower control limits for internal controls used in the assay for IQC, OD of positive and negative controls and PI of positive and negative controls are given in Table 1.

Table 1. Internal Quality Control Data

IQC	LCL	UCL
OD CC	-0.05	0.07
OD C-	0.65	1.34
PI C++	94.26	102.8
PI C-	-4.26	4.33

**LCL = Lower Control Limits, UCL = Upper Control Limit; PI = Percentage Inhibition; OD = net Optical Density Value*

3.8 Data analysis

The questionnaire data concerning animal, management, environmental factors collected from the farmers electronical via tablet, were uploaded via the internet to the project's data base for storage. Animal data together with the laboratory results were recorded, and stored on a Google sheet where only authorized individuals had access to it. All data were later captured in a Microsoft Excel® spread sheet and cleaned before it was transferred into Stata 14 (StataCorp, College Station, TX, U.S.A.) for analysis.

Separate analyses were performed for cattle and for goats. An animal was defined as seropositive to RVFV if it tested positive using both the i-ELISA and the inhibition ELISA. Overall seroprevalence was calculated, as well as age-, district- and breed- specific seroprevalences, with 95% confidence intervals. Using the "*svy: proportion*" command in Stata 14, the prevalence was adjusted by weighing each observation by the inverse of the sampling fraction. The estimates and confidence intervals were adjusted to account for the multi-stage sampling design and variation in sampling weights between farms. Sample weight, also called probability weight, is the inverse of the likelihood of being sampled or the inverse of the sampling fraction. When different sampling methods are applied to subgroups within the population studied, sampling weights are used to restore the original importance of each group within the population to reduce unintended sample bias arising during the process of conducting the survey and to ensure that the sample is representative of the population of interest (Kalsbeek et al. 2010).

The sample weight was calculated as $\frac{N}{n}$ where N = herd size and n = sample size or it can be calculated as $\frac{1}{sample\ fraction(\frac{n}{N})}$

To estimate associations between the potential risk factors and seroprevalence, each factor was assessed by cross-tabulation using 2×2 contingency tables and odds ratios were calculated. Statistical significance was set at $P < 0.05$ using Fisher's exact test.

A univariate analysis using contingency tables was done to select variables for inclusion into the multiple logistic regression model. Fisher's exact test *P*-value was reported and $P < 0.2$ was used as an inclusion criterion into the model in other to identify important variable as recommended by (Budtz Jorgensen et al. 2006) when backward elimination is used.

Multivariable analysis was done using multilevel logistic regression models and a backward elimination procedure was performed, since all set of selected variables are included in the model it is less likely to omit a confounding variable from the model (Sun et al. 1999). A *P*-value less than 0.05 ($P < 0.05$) was used as the criterion for retention in the model. The variable with the largest *P*-value was removed out of the model and it was then re-run. This was done subsequently until all the remaining variables were significant at $P < 0.05$ in the final model. Then all variables were individually re-tested in the model and retained if significant. The fit of the final models, excluding the random effects, was assessed using the Hosmer-Lemeshow goodness-of-fit test.

For each species (cattle and goats), seroprevalence was calculated for each farm on which three or more animals were sampled. These within-farm seroprevalences, with their geographic co-ordinates, were then imported into a GIS programme (ArcGIS 10.2; ESRI, Redlands, CA, U.S.A.) and a raster of predicted intra-farm seroprevalence over the entire study area was produced by interpolation using inverse distance weighting.

CHAPTER 4

4.1 RESULTS

A total of 956 cattle and 520 goats were sampled from 83 and 52 farms respectively. Overall RVF crude seroprevalence was 25.5% (244/956) in cattle and 7.5% (39/520) in goats.

After the estimates were adjusted for clustering and sampling weights, RVF seroprevalence was estimated to be 30.5% (95% CI: 24.6-37.0%) for cattle and 8.8% (95% CI: 4.1-18.1%) for goats. In the concurrent study on sheep, the estimated seroprevalence was 14.2% (95% CI: 9.7-20.3%). During sampling, some farmers decline to participate in the survey and no apparent RVF clinical signs were observed on the farms during the survey.

Three age categories were used for this study, <2 years, 2-4 years (born since the last outbreak), and >4 years (alive during the last outbreak 2011). The age category with the highest seroprevalence was in the age category of >4 years, i.e. those alive during the last outbreak in 2011, with 46.4% and 16.7% for cattle and goats respectively. The lowest seroprevalence was observed in the <2 years age category for both species with cattle having 2.6% and goats 1.1% (Tables 2 and 3).

A total of 824 females were sampled with 28.5% seropositive and 131 males with 6.9% seropositive in cattle, whereas in goats 442 females were sampled with 8.6% seropositive and 78 males were sampled with 1.3% seropositive.

The significance of the univariate associations between RVF IgG seropositive results and potential risk factors, are shown in Tables 2 and 3 for cattle and goats, respectively.

Table 2. Univariable analysis of potential risk factors for seropositivity to RVF in cattle

Risk factor and level	Number of animals sampled	Number testing positive	% seropositive	*P*-value[†]
District*				<0.001
Barkley West	26	3	11.5	
Bloemfontein	179	48	26.8	
Boshof	157	55	35.0	
Brandfort	38	19	50.0	
Bultfontein	69	30	43.5	
Edenburg	43	15	34.9	
Fauresmith	51	13	25.5	
Herbert	14	4	28.6	
Jacobsdal	39	10	25.6	
Jagersfontein	28	1	3.6	
Kimberley	89	9	10.1	
Koffiefontein	5	1	20.0	
Petrusberg	92	25	27.2	
Philippolis	18	2	11.1	
Reddersburg	27	3	11.1	
Smithfield	25	1	4.0	
Trompsburg	41	5	12.2	
Warrenton	6	0	0.0	
Breed				0.241
Cross	415	111	26.7	
Exotic	330	79	23.9	
Indigenous	92	30	32.6	
Age*				<0.001
<2 years	195	5	2.6	
2-4 years	275	14	5.1	
>4 years	483	224	46.4	
Animal born on farm				1.000
No	228	53	23.2	
Yes	588	136	23.1	

Sex*				<0.001
Female	824	235	28.5	
Male	131	9	6.9	
Vaccinated against RVF*				0.155
No	426	107	25.1	
Yes	163	51	31.3	
Unknown	367	86	23.4	
Type of farm*				0.001
Communal	57	4	7.0	
Private	899	240	26.7	
Mixed with other animals				0.765
No	417	104	24.9	
Yes	539	140	26.0	
Kept at night*				0.001
Kraal	156	18	11.5	
Veld	692	204	29.5	
Both	75	12	16.0	
Do farm animals have contact with other species				0.559
No	788	198	25.1	
Yes	168	46	27.4	
Access to permanent dam(s) (natural water source)*				0.002
No	550	160	29.1	
Yes	374	75	20.1	

Access to stream(s)/river(s) (constantly flowing water source)				0.515
No	645	168	26.0	
Yes	279	67	24.0	
Access to seasonal pan on farm (water source)*				<0.001
No	542	99	18.3	
Yes	382	136	35.6	
Access to dam(s) (manmade water source)*				0.107
No	205	61	29.8	
Yes	719	174	24.2	
Canal on farm (water source)				0.746
No	938	240	25.6	
Yes	18	4	22.2	
Borehole on farm (water source)*				0.007
No	928	243	26.2	
Yes	28	1	3.6	
New animals brought onto farm in past 12 months				0.945
No	566	144	25.4	
Yes	390	100	25.6	
Biosecurity*				0.032
No	349	103	29.5	
Yes	607	141	23.2	

Farm size tercile (ha)*				0.026
<845	291	60	20.6	
846 - 2500	361	108	29.9	
>2500	296	76	25.7	
Main industry*				0.012
DairyMeat	42	16	38.1	
Resale	118	30	25.4	
Other	190	35	18.4	
Production system*				0.002
Cash sales	115	12	10.4	
Commercial	633	186	29.4	
Feedlot	84	19	22.6	
Semi commercial	124	27	21.8	
Animals slaughtered on farm*				0.011
No	376	117	31.1	
Yes	523	123	23.5	
Year of last RVF vaccination on farm*				0.004
Never	546	114	26.4	
2009-2011	112	22	19.6	
2012-2013	67	10	14.9	
2014-2016	118	45	38.1	
Unknown	113	23	20.4	

†*P*-Value for Fisher's exact test.

*Variable significant ($P < 0.20$) for inclusion into multiple logistic regression model

Table 3. Univariable analysis of potential risk factors for seropositivity of RVF in goats

Risk factor and level	Number of animals sampled	Number testing positive	% seropositive	*P*-value[†]
District*				0.028
Barkley West	82	0	0.0	
Bloemfontein	69	8	11.6	
Boshof	63	10	15.9	
Edenburg	3	0	0.0	
Fauresmith	41	2	4.9	
Herbert	18	1	5.6	
Hopetown	6	0	0.0	
Jacobsdal	36	4	11.1	
Jagersfontein	9	2	22.2	
Kimberley	111	4	3.6	
Koffiefontein	35	4	11.1	
Petrusberg	6	0	0.0	
Philippolis	32	3	9.4	
Trompsburg	8	0	0.0	
Breed				0.614
Exotic	51	3	5.9	
Indigenous	445	35	7.9	
Age*				<0.001
<2 years	184	2	1.1	
2-4 years	129	3	2.3	
>4 years	203	34	16.7	
Animal born on farm				0.636
No	98	7	7.1	
Yes	371	32	8.6	
Sex*				0.024
Female	442	38	8.6	
Male	78	1	1.3	

Vaccinated against RVF*				0.009
No	274	19	6.9	
Yes	49	9	18.4	
Unknown	197	11	5.6	
Type of farm*				<0.001
Communal	192	2	1.0	
Private	328	37	11.3	
Mixed with other animals				0.212
No	165	16	9.7	
Yes	355	23	6.5	
Kept at night*				<0.001
Kraal	286	10	2.5	
Veld	225	29	12.9	
Both	9	0	0.0	
Do farm animals have contact with other species				0.247
No	357	30	8.4	
Yes	163	9	5.5	
Access to permanent dam(s) (natural water source)*				0.178
No	369	24	6.5	
Yes	151	15	9.9	
Access to stream(s)/river(s) (constantly flowing water source)*				<0.001
No	376	17	4.5	
Yes	144	22	15.3	
Access to seasonal pan on farm (water source)				0.298
No	371	25	6.7	
Yes	149	14	9.4	

Access to dam(s) (manmade water source)				0.682
No	237	19	8.0	
Yes	283	20	7.1	
Canal on farm (water source)*				0.004
No	519	38	7.3	
Yes	1	1	100.0	
Borehole on farm (water source)				0.617
No	505	39	7.7	
Yes	15	0	0.0	
New animals brought onto farm in past 12 months*				0.001
No	404	22	5.4	
Yes	116	17	14.7	
Biosecurity*				0.117
No	289	17	5.9	
Yes	231	22	9.5	
Farm size tercile (ha)				0.232
<845	189	20	10.6	
846 - 2500	148	8	5.4	
>2500	133	11	8.3	
Main industry				0.253
DairyMeat	198	26	13.1	
Resale	73	8	11.0	
Other	57	3	5.3	
Production system*				0.001
Cash sales	252	7	2.8	
Commercial	189	23	12.2	
Feedlot	9	1	11.1	
Semi commercial	70	8	11.4	

Animals slaughtered on farm*				0.003
No	107	4	3.7	
Yes	221	33	14.9	
Year of last RVF vaccination on farm*				0.015
Never	373	24	6.4	
2009-2011	57	11	19.3	
2012-2013	29	1	3.5	
2014-2016	27	0	0.0	
Unknown	34	3	8.8	

†*P*-Value for Fisher's exact test.

*Variable significant ($P < 0.20$) for inclusion into multiple logistic regression model

For cattle and goats, several potential risk factors for seropositivity were significant at $P < 0.20$ in the univariate analyses. These included district, age, sex, RVF vaccination status of an animal, communal/private grazing land, when was the most recent RVF vaccine given, biosecurity (this is where animals are in quarantine, vaccinated, dipped or giving antibiotic treatment before introduced in to the herd), animals slaughter on farm and, production system. For cattle, specific risk factors included where animals kept at night, access to seasonal pan on farm (water source), man-made dam water source on the farm, and borehole water source on the farm were significant at $P < 0.20$. For goats, specific risk factors included farms where animals mixed with other farms to graze, presence of permanent dam, presence of permanent river and new animal purchased or brought into the farm in the past 12 months were also significant at $P < 0.20$. These factors were then included into multilevel logistic regression models; the final models are shown in Tables 4 and 5 for cattle and goats, respectively. Note that, because of the large number of levels, district was included in the model as a random effect rather than a fixed effect, and the random effect for farm was nested within district.

Table 4. Final multilevel logistic regression model of factors associated with seropositivity to RVF in cattle in the study area

Risk factor and level	OR	95% CI	*P*-value
Age (years)			
<2	1*		
2-4	3.01	0.83-11.0	0.094
>4	94.0	27.2-325	< 0.001
Kept at night			
Kraal	1*		
Veld	9.02	2.56-31.85	0.001
Both	5.06	1.18-21.68	0.029
Access to seasonal pan on farm (water source)			
No*	1*		
Yes	3.78	2.1-6.79	<0.001
Main industry			
Meat*	1*		
Dairy	12.8	2.73-59.86	0.001
Resale	0.76	0.31-1.82	0.532
Others	0.48	0.22-1.05	0.065
Year of last RVF vaccination on farm			
Never*	1*		
2009-2011	0.65	0.27-1.53	0.321
2012-2013	0.25	0.08-0.79	0.018
2014-2016	3.83	1.53-9.64	0.004
Unknown	1.29	0.53-3.14	0.576

Significance of random effects for district and farm: $P = 0.0002$

Hosmer-Lemeshow chi-squared (8 d.f.) = 8.81 ($P = 0.359$)

*Reference category

OR = odds ratio, CI = confidence interval

The final logistic regression model showed that in cattle, odds of seropositivity increased with age and was higher in the >4 years category. The odds of seropositivity was high in cattle not kraaled at night and those that are either kraaled or kept in veld at night as compared to those kraaled at night only. It was also observed that the odds of seropositivity was high in farms that has access to seasonal pan and in dairy cows, as well as in cattle vaccinated against RVF between 2012-2013 and 2014-2016 (Table 4).

Table 5. Final multilevel logistic regression model for factors associated with seropositivity to RVF in goats in the study area

Risk factor and level	OR	95% CI	*P*-value
Age (years)			
<2*	1*		
2-4	1.48	0.22-9.82	0.685
>4	16.7	3.66-76.53	< 0.001
Type of farm			
Communal*	1*		
Private	29.0	4.59-182.73	<0.001
Access to seasonal pan on farm (water source)			
No*	1*		
Yes	7.69	1.54-38.48	0.013
Access to stream(s)/rivers(s) (constantly flowing water source)			
No*	1*		
Yes	24.8	4.72-129.94	<0.001
Access to permanent dams(s) (natural water source)			
No*	1*		
Yes	4.18	1.09-16.00	0.036
Access to dams(s) (manmade water source)			
No*	1*		
Yes	0.10	0.024-0.43	0.002

Significance of random effects for district and farm: $P = 0.0002$

Hosmer-Lemeshow chi-squared (8 d.f.) = 13.89 ($P = 0.085$)

*Reference category

OR = odds ratio, CI = confidence interval.

The final logistic regression model for goats showed that, the odds of seropositivity increase among animals older than four years of age (born before the most recent outbreak) and 2-4 years of age category as compared to the <2 years category. The odds of seropositivity was high in goats that graze on private grazing land compared to those that graze on communal land, and was also highly associated with the presence of seasonal pan water source, access to stream(s)/river(s) (constantly flowing water source on the farm), and access to permanent dam(s) (natural water source), on the farms. However, the odds of seropositivity was negatively associated with the presence of manmade water source on the farms (Table 5).

Geographic information system software was used to map the distribution of seroprevalence in the study area using inverse distance weighting.

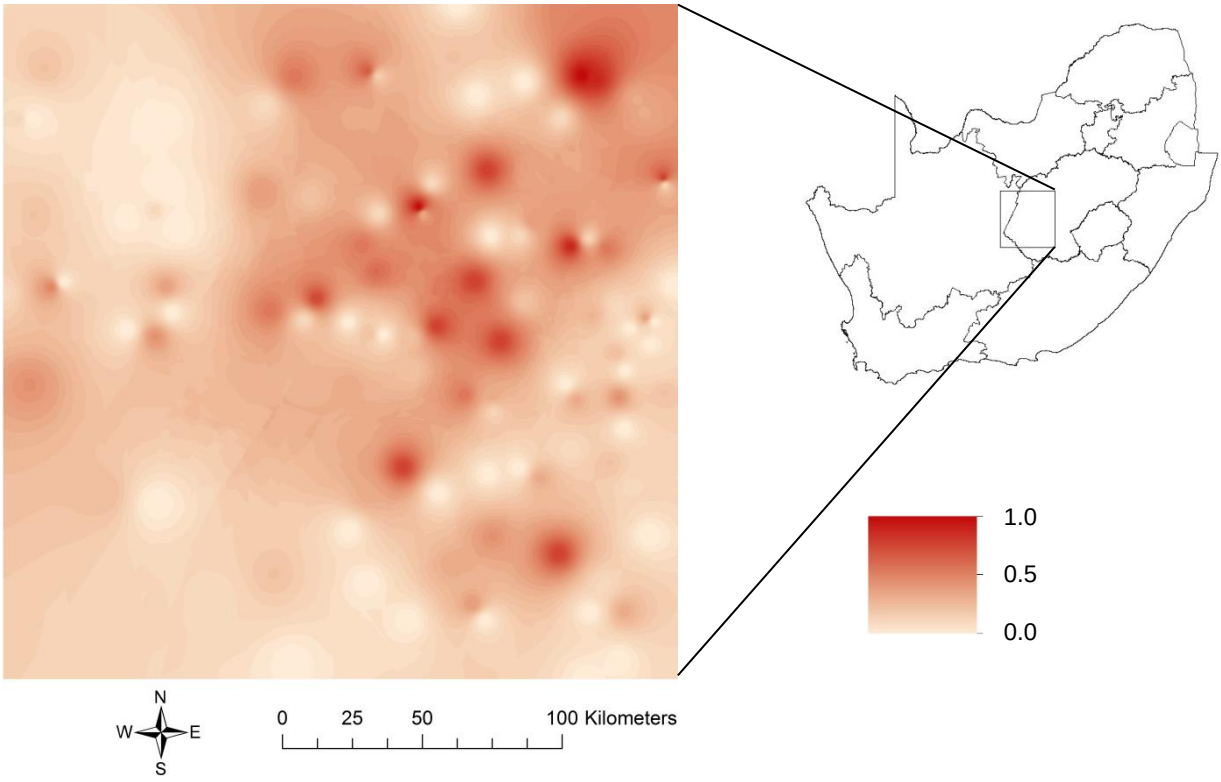

Figure 6. Distribution of seroprevalence of RVF in cattle in the study area from September 2015-January 2016.

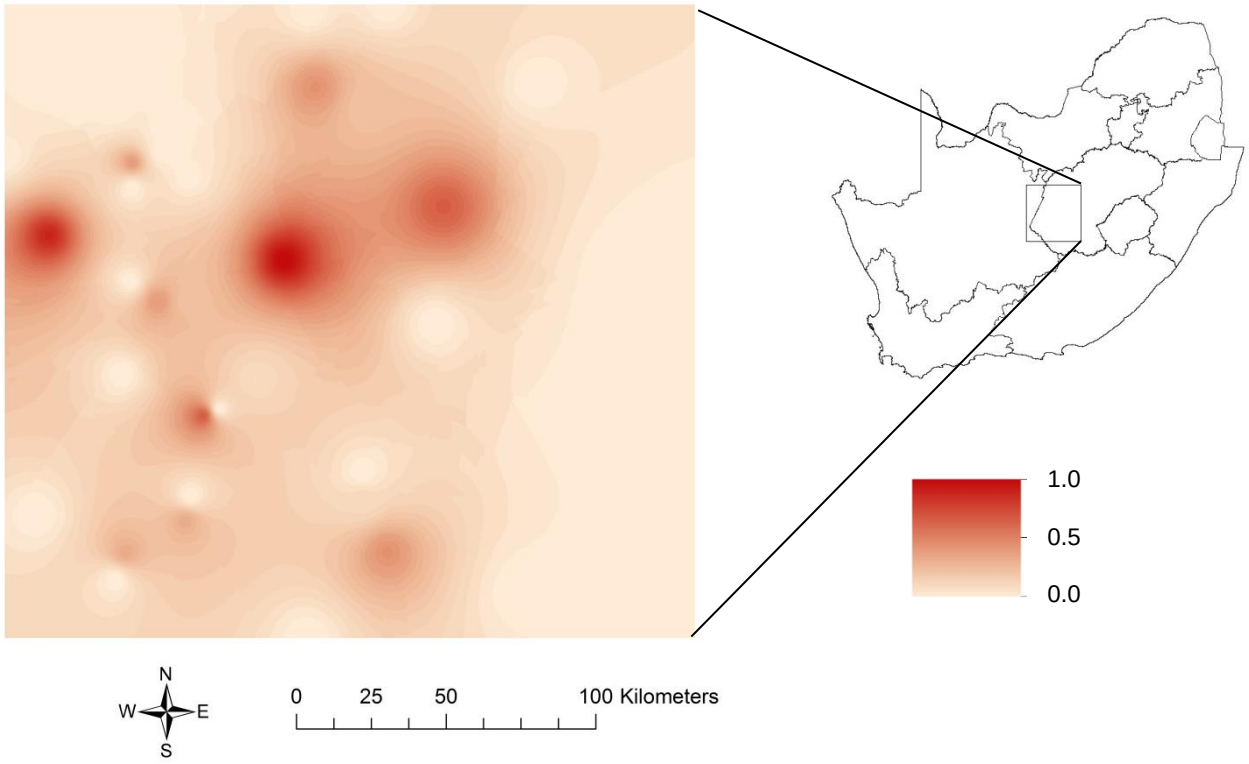

Figure 7. Distribution of seroprevalence of RVF in goats in the study area from September 2015-January 2016

Figures 6 and 7 show the distribution of percentage seroprevalence at farm level for cattle and goats respectively. The seroprevalence increases from south-west to north-east in cattle, whereas in goats the distribution shows isolated areas of high seroprevalence in central and western area of the study area.

Chapter 5

5.1 DISCUSSION

This study has shown how feasible it is to conduct a large-scale survey for disease investigation using random geographic point sampling by plotting points on Google Earth. The state veterinarians were very resourceful; they provided maps of all the farms within the study area, contact phone numbers of the farmers and linked the researchers with veterinarians and health technicians in charge of the districts. This made it very easy for the researchers to locate and contact farmers for possible appointment. It can therefore be learned from this study that plotting sampling points on Google Earth makes it easy to travel and locate the farms. Stakeholder's meetings, posters and banners were very important in explaining the project to the farmers and farm managers and inviting them to participate in the study. The questionnaire was translated into local languages for easier understanding by the farmers and the farm managers

A few challenges were encountered during this study, with some farmers declining to participate, some farm owners were not living on the farms, abandoned farms, and animals let out of kraal to graze before the arrival of researchers on the farms. However, the required number of animals was achieved.

The study has provided evidence of seropositivity to RVF in domestic cattle and goats in some locations of the study area and they were associated with some risk factors. However, it was not possible to distinguish whether seropositivity was due to vaccination or natural infection of RVFV. This is because many farmers could not remember if the animals were vaccinated against RVFV, and have no vaccination record book, in which animal's identification number, vaccination date, year and type of vaccine administered can be recorded.

This study estimated the overall individual animal seroprevalence of RVF in cattle and goats to be 30.5% (95% CI: 24.6-37.0%), and 8.8% (95% CI: 4.1-18.1%) respectively. Previous studies have documented similar seroprevalence of 33.3% in 12 cattle but a higher value of 25.8% in 271 goats from Garissa county of Kenya in a cross-sectional study conducted in July 2013 (dry season) (Nanyingi et al. 2016). In addition, result of this study is slightly higher compared with seroprevalences of 27.7% from 756 cattle but lower compared with the 20.2% from 531 goats reported from cross sectional study conducted between April and August 2013, six years after the 2006/2007 RVF outbreak in Tanzania (Sindato et al. 2015).

A study reported during the dry season (April – August 2009) from the Republic of Comoros has earlier reported a seroprevalence of 30.6% from 196 cattle and 33.5% from 84 in goats (Roger et al. 2011). However, the results from this study was higher compared with both 7.8% from 727 cattle and 5.0% from 219 in goats reported recently in a two-phase prospective cross-sectional study conducted between November 2010 and November 2012 from Central African Republic (Nakouné et al. 2016).

The difference in seroprevalence between cattle and goats recorded in this study might be attributed to vector preference for cattle compared to goats, difference in age and immune response, genetic background, differences in management system (require further study) or species susceptibility; this is similar to the findings reported in Kenya (Rostal et al. 2010), Rwanda (Umuhoza et al. 2017), Republic of Comoros (Roger et al. 2009) and Mozambique (Blomstron et al 2016). Other studies have also reported lower seroprevalence in goats than cattle and sheep (Chevalier 2013, Nabeth et al. 2001).

Other possible reasons for the higher seroprevalence observed in cattle than goats may be because cattle are kept for a longer period by farmers than goats, providing opportunity for RVFV seroconversion or because farmers have more preference and value for cattle than goats, therefore, may be more likely vaccinate cattle than goats.

This study showed that seroprevalence varied by age. The highest seroprevalence was found in animals older than four years in both species. Therefore, the older the animals the higher the opportunity of being infected. This may be related to the fact that they survived the last outbreak, and stood the chance of being more exposed to RVFV. The higher seroprevalence in older animals indicates that long time exposure is a risk factor, and this is commonly seen in most infectious diseases. This finding agrees with recent studies conducted in Rwanda (Umuhoza et al. 2017) and Central African Republic (Nakouné et al. 2016). The significant seropositivity found in cattle born since the last outbreak (2-4 years age category) indicates that subclinical RVFV circulation might have occurred in the relevant areas, a similar situation has been previously reported in 2006 in Saudi Arabia (Davies et al. 2006).

The high seroprevalence in cattle not kraaled at night indicates that natural infection may have occurred, since it is consistent with what is known about this environmental risk factor for infection (Gargan, Jupp & Novak 1988). A possible explanation for the high seroprevalence in cattle not kraaled at night could be that, they are more likely to be exposed to mosquitoes at night and graze where numerous mosquitoes vectors breed, this is in

accordance with a previous report that *Aedes*, *Culex* and *Eretmapodites* are the main RVFV epizootic vectors found in this study area (McIntosh et al. 1980).

This study noted high odds of seropositivity among cattle that have access to seasonal pans (water source) on the farm; this may possibly be attributed to transovarial transmission of RVFV from the eggs of *Aedes* spp. (Linthicum et al. 1985) as well as transmission by other species of mosquito that breed around the pans. We also observed a high seroprevalence in dairy cattle compared to other industries. This could be because dairy cattle are more likely to be vaccinated, with farmers knowing that even mild or subclinical RVF infection can affect their production.

Cattle vaccinated between 2014-2016 showed increased odds of seropositivity compared to those vaccinated in the previous years, indicating the possible presence of high antibody titers due to vaccination. However, vaccination history may sometimes not be reliable, as some farmers were more likely to remember recent vaccination more accurately. This can also be associated to the type of vaccine used. Smithburn RVF vaccine has been reported to evoke long lasting antibody, however level of herd immunity decrease with increase in years after administration of the vaccine and percentage of IgG antibody decreases from 95% to 66.7% after 1 year, and up to zero after 6-7 years and have been reported to induce low antibody titres in cows (Alhaj et al. 2016, Elfadil 2007). It could also be associated to whether the cattle were vaccinated with Clone 13 RVF vaccine; this vaccine seroconversion persists for 1 year after vaccination (Lo, Modou et al. 2015). Formalin-inactivated RVF vaccine has been reported to induce high antibody titres and evoke protection for 9 months in cattle, and up 2 years if a booster is given after 3 months (Barnard et al. 1977, Langerqvist et al. 2012).

Goats pastured on private grazing land showed higher seropositivity compared to those reared on communal land. Vaccine induced seropositivity is a likely factor that could influence seropositivity; since vaccination is not compulsory, private farmers are probably more likely to vaccinate their herds compared to communal farmers who are less informed about the economic importance of this virus. It may also possibly be attributed to the presence of permanent river(s)/stream(s), seasonal pans, access to permanent dams (natural water source) and access to dams (man-made water source) on private lands that potentiates mosquitoes breeding. It is possible that private farms may have been situated in areas more suitable for disease transmission. However, these factors were included in the multivariable model and their potential confounding effect should have been removed.

Other risk factors that may influence seropositivity is the possible contamination of aborted fetal material during grazing, slaughtering of subclinical infected animals on the farms, bull sharing among farmers, and introduction of animals purchased/brought in to farms without quarantine and vaccination (Bird et al. 2011, Archer et al. 2013, and Chevalier 2013). The introduction of animals purchased/brought into new location has been reported as the route through which RVFV entered Egypt during the 1977 outbreak (Gad et al. 1986). Also in Saudi Arabia and Madagascar, it has been reported that animal movement and trade from infected region is a possible risk factor of RVFV transmission to a new environment (Mohamed et al. 2014, Lancelot et al. 2017).

This study shows that RVFV seroprevalence varies by location; the highest seroprevalence was found in the district of Brandfort for cattle and Jagersfontein for goats. The district with the lowest seroprevalence was Jagersfontein for cattle and Kimberly for goats. There was no seropositivity recorded in the district of Warrenton for cattle and district of Barkley West, Edenburg, Hopetown, Petrusburg and Trompsburg for goats. The difference in districts can be attributed to several factors, including differences in breeds and species of livestock reared in the districts, local agro-ecological conditions, water sources, presence of stagnant water pans/dams in some districts, availability of mosquito vectors (Bousini et al. 2014, Fafetine et al 2013). This potentiates the hatching of transovarially infected *Aedes* spp. in flood water and initiate transmission of the virus (Metras et al. 2015). High environmental temperature that favors the breeding of mosquitoes has been shown to be consistent with RVF outbreak in Kenya (Woods et al. 2002, Lichoti et al. 2014).

Another possible factor for the difference in seroprevalence in districts could be the type of farming system practice by the farmers. Some farmers practice mixed farming by rearing livestock and growing crops especially rice on the same farm land, this provides a suitable breeding ground for mosquitoes and have been linked to a RVF outbreak in Republic of Comoros (Chevalier et al. 2011).

Although not statistically significant ($P < 0.2$), the prevalence of RVF was lower in exotic cattle (23.9%) when compared to cross and indigenous breed, and goats (5.9%) when compared to indigenous breed. This can be attributed to management systems where farmers tend to manage the indigenous breeds extensively compared to exotic breeds. However, this requires further study including adjustment for other possible confounding variables to determine breed susceptibility.

Also, not significant ($P < 0.2$), this study recorded seroprevalence in cattle and goats that mixed with other animals. Similarly, also not significant this study recorded seroprevalence in cattle and goats that has contact with other species. The mixing of animals most especially with wildlife has been reported to plays an important role in RVFV transmission (Britch et al. 2013, Evans et al. 2008, LaBeaud et al. 2011).

There was difference in seroprevalence by sex for both species, for goats' sex was statistically significant at ($P < 0.02$) with seroprevalences of 8.6% and 1.3% in females and males respectively. For cattle, the differences were significant ($P < 0.001$) with seroprevalences of 28.5% and 6.9% in females and males respectively. However, when included in the multivariable model,sex was no longer significant. Similarly, a study of RVF seroprevalence in livestock reported a slightly lower seroprevalence of 26.0% in females and slightly higher 7.8% in males in a cross sectional study conducted during inter-epidemic period in Tanzania (Sumaye et al. 2013). The cattle results from this study observed higher seropositivity compared to not statistical significant difference of 11.2% in male and lower compared to 18% in females reported in cattle from a study conducted in Akagera-Nyabarongo Rwanda (Umuhoza et al. 2017). Some studies have reported no statistical difference in sex with 4.5% in females and 8.2% in males and in livestock from Kigoma district of Tanzania (Kifaro et al. 2014). 15.7% in females and 2.9% in males in a serological study conducted in Zambezia province of Mozambique (Fafetine et al. 2013). Therefore, it is very difficult to conclude that sex is a risk factor and the apparent difference between sexes was likely due to confounding by other variables.

Individual vaccination status of animal against RVF for both cattle and goats were significant at ($P < 0.2$) for cattle and ($P < 0.05$) for goats during univariable analysis but were both left out in the final multivariable logistic regression model, because they were both not significant at ($P < 0.05$). This can be linked to the fact that antibody decreases after several years of vaccination as well as possibly unreliable vaccination records, improper vaccination handling and administration as discussed earlier.

5.2 Limitations of the study

There were several possible sources of bias in this study. Random sampling was not possible at some farms; animals were preferentially selected by the farm owners for sampling. Therefore, samples collected from such farms were not representative of the population of animals on the farms and might have led to possible systematic error. Bias could have occurred in situations where farmers declined to participate in the survey or there were no animals on the random geographic points generated, the next farm was selected based on convenience not far from the originally generated point.

Another possible way bias could have also occurred when sampling according to age category (three animals per category), where the number of animals was not up to three in each category more animals from other age group were sampled to complete the desired nine animals per farm. While in situation where there were less than nine animals on the farm, all the available animals were sampled. Therefore, to minimize such bias in the overall seroprevalence, the data were adjusted to account for clustering and sampling weight.

The questionnaire was designed as closed-ended, therefore, there is possibility of responding to wrong question, could suggest ideas that farmer is not aware, there are possibility that respondents misinterpreted some questions and that cannot be identified by the researcher. Also, due to the magnitude of the study the questionnaire was administered by more than one person, therefore some questions may have been explained differently to the respondents. Another possible source of bias was that some respondents got tired due to the length of the questionnaire and may have selected answers just to get finished. Samples collected towards the middle of summer (January) might have biased the results, because this period marks the onset of perfect breeding conditions for water *Aedes* mosquitoes.

The result from this study may not be representative for the whole country, especially where the geographic ecosystems differ, because it only covers two provinces. This study has recorded wide variation in the seroprevalences in different districts with diverse vaccination practices and local agro-ecological conditions that are possible risk factors to the seropositivity recorded. There is need to extend this study to all part of the country that had previous history of RVF outbreak. This will give a more complete picture of risk factors and seropositivity of the virus for the country.

This study only tested for IgG antibody and positive results were further confirmed using inhibition ELISA. Sera were not tested for IgM which test for recent exposure and VNT which is the gold standard to account for false positive. The ideal was to test for IgM, IgG, and VNT but due to budgetary constraints and time it was not possible. Since IgM testing was not conducted, information on recent RVF infection was not obtained. The diagnostic test used in this study could not differentiate between seropositivity due to vaccination or infection (DIVA).

CHAPTER 6

6.1 Conclusions

Seroprevalence was higher in cattle 30.5% (95% CI: 24.6-37.0%) than in goats 8.8% (95% CI: 4.1-18.1%), but in both species the level of herd immunity four years after the last reported outbreaks was low. The presence of significant level of RVF IgG antibody against RVFV in the study area among cattle not born during the last outbreak (2-4 years category), associated with known environmental risk factors for RVF transmission indicate the possibility that viral circulation might have taken place during the inter-epidemic period without showing any clinical signs in the animals.

This study was important in providing knowledge on RVF seroprevalence and associating risk factors that will provide ideas in designing national RVF control program and policy making.

It is evident from this study that RVFV antibodies were detected in all the 18 districts of the study area with both communal and private management system, except Warrenton. However, this study could not ascertain seroprevalence due to vaccination or due to natural circulation. Nevertheless, there is need for further study and to increase RVF awareness among farmers and farm workers regarding zoonotic risks associated with slaughtering animals on farms and contact with aborted fetal materials and to implement appropriate control measure.

Vaccination of animals against RVF, and quarantine of newly purchased/animal brought to the farm is highly recommended before they are allowed in to the herd.

Using one health approach, veterinarians, public health professionals and ecologist should work together to ensure early prediction and detection of RVF outbreak in both humans and animals.

6.2 Areas for further research

Due to the public health, epidemiological and economic importance of RVF in South Africa, a cohort study to determine recent infections and to predict possible future outbreaks is essential, as well as to detect whether and where inter-epidemic circulations takes place.

The difference among districts need to be explained, therefore there is need for further study to understand the possible risk factors associated with RVF and RVFV exposure in different districts.

There is need to isolate and characterize the circulating RVFV to identify the viral strains circulating in the study area and to determine whether the virus differs between the epizootic and inter-epizootic periods.

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Appendix A - Questionnaire

DEMOGRAPHIC INFORMATION

Owners detail:

Name: _____

Farm details:

Farm name_____

Farm district: _____

Farm size_____

Farm coordinate_____

Farm system:

Feedlot [ ] Extensive [ ] others (specify) _____

Types of animals on farm and their numbers

Species	**Numbers**
Cattle	
Sheep	
Goat	
Wild Ruminants (specify)	
Others (specify)	

Have there been new animal(s) introduce to the farm? Yes [ ] No [ ]

If yes, please fill appropriately

How many animals introduce into the farm?			
This year	Last year	In the last two years	In the last three years and above

Has there been contact of cattle with other animals? Yes [ ] No [ ]

If yes, please select the appropriate box

Goats: On daily basis [ ] weekly [ ] monthly [ ] occasionally [ ]

Sheep: On daily basis [ ] weekly [ ] monthly [ ] occasionally [ ]

Wild Ruminants : On daily basis [ ] weekly [ ] Monthly [ ] occasionally [ ]

Other(s)……On daily basis [ ] weekly [ ] Monthly [ ] occasionally [ ]

…….On daily basis [ ] weekly [ ] Monthly [ ] occasionally [ ]

Has there been contact of goats with other animals? Yes [ ] No [ ]

If yes, please select the appropriate box

Cattle: On daily basis [ ] weekly [ ] Monthly [ ] occasionally [ ]

Sheep: On daily basis [ ] weekly [ ] Monthly [ ] occasionally [ ]

Wild Ruminants: On daily basis [ ] weekly [ ] Monthly [ ] occasionally [ ]

Other(s)……On daily basis [ ] weekly [ ] Monthly [ ] occasionally [ ]

…….On daily basis [ ] weekly [ ] Monthly [ ] occasionally [ ]

ENVIRONMENT

Source of water for animals

Borehole [ ] Man-made dam [ ] River [ ] Vlei/Pan [ ]

Which water source do cattle and goat have access to?

Borehole [ ] Man-made dam [ ] River [ ] Vlei/Pan [ ]

Are the source of water on your farm

Permanent [ ] Seasonal [ ] Other(s)……

Management:

Where do the cattle and goats normally sleep?

Open field [ ] **Roofed pen** [ ] **Pen without roof** [ ]

How often do they sleep there?

Open field: Every night [ ] weekly [ ] Monthly [ ] seasonally [ ]

Roofed pen: Every night [ ] Weekly [ ] Monthly [ ] seasonally [ ]

Other(s)……Every night [ ] Weekly [ ] Monthly [ ] seasonally [ ]

Vaccination History

How often do you vaccinate your animals against Rift Valley fever?

Please select the appropriate box

Never [ ] occasionally [ ] during outbreaks [ ] annually [ ]

If vaccination occur, who vaccinate your animals?

Self [ ] Labourers [ ] Veterinarian [ ]
Other(s)……

Which of your animal species do you vaccinate against Rift Valley Fever?

Cattle [ ] Sheep [ ] Goat [ ] wild Ruminants [ ]
other(s)……
…

When did you last vaccinate against Rift Valley Fever?

6months to 2 years ago [ ] 2 to 4 years ago [ ] Greater than 4 years [ ]

Which group of cattle were vaccinated?

Young cattle [ ] New animals [ ] Most valuable animals [ ] Bulls only [ ]
Cows only [ ] other(s)….....

Which group of goats were vaccinated?

Kids [ ] New animals [ ] Most valuable animals [ ] Bucks only [ ] Does only [ ] other(s)….....

Which product of vaccine do you use?

Smithburn vaccine [ ] Inactivated vaccine [ ] Clone 13 [ ] other(s) [ ]

What type of feeding system do you use?

Veld [ ] Supplementary feed [ ] Feedlot [ ] other (s) [ ]

How often have you seen the following in your flock?

Abortions	Never	Past year	1-4 years ago	>4 years ago
Frequency:	Normal		Above normal	
Stillbirths	Never	Past year	1-4 years ago	>4 years ago
Frequency:	Normal		Above normal	
Mortalities in Calve/kids(before weaning)	Never	Past year	1-4 years ago	>4 years ago

Frequency:				
	Normal		Above normal	
Mortalities in adult cattle/goats	Never	Past year	1-4 years ago	>4 years ago
Frequency:	Normal		Above normal	

Appendix B - Consent Form

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Seroprevalence and Risk Factors for Rift Valley Fever in cattle and goats in the Free State and Northern Cape provinces

Main investigator: Dr Yusuf B Ngoshe

Supervisor: Prof Peter Thompson

INFORMED CONSENT FORM

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

1. Project details:

• BACKGROUND & PURPOSE OF RESEARCH PROJECT:

Rift Valley fever is a disease that is seen in outbreaks after periods of abnormally high rainfall. It affects all livestock species and causes abortion storms with high numbers of deaths especially in new-born and young animals. It may also affect adult animals and signs of weakness, anorexia, nasal discharges, diarrhoea and even death may be seen. The virus can also infect humans when they come in contact with infected animal tissues, drink infected milk or get bitten by an infected mosquito. Flu-like signs may be seen. Although rare, complications can also occur in human cases where hepatitis, kidney failure, meningioencephalitis, blindness and death may occur.

Because this disease is so important in our domestic livestock as well as humans in contact with these animals, we need to be able to understand how this disease works.

For this study, we want to determine the antibody levels in cattle and goats against this virus in a period between outbreaks. This will help us to determine the normal protection level in

the cattle and goat's population and different age groups of animals. It may also help us find out how the disease survives between outbreaks and identify risks that may be associated with presence of the disease.

• DETAILED PROCEDURE(S) TO BE PERFORMED:

Your farm was selected randomly, together with about 204 other farms to take part in this study. Nine cattle and goats each will be selected from your herd/flock. We will divide the cattle and goats into three age groups and select three animals per age group (if possible). Details of each cattle will be recorded e.g. breed, age, sex, identification, vaccination status, etc. One blood sample will then be drawn from each of these selected animals and they will be released back to the flock again thereafter.

If permitted, we will require your help or that of your farm laborer to assist us in restraining the sheep so that we can draw blood from the jugular vein in the neck or tail vein. We will give proper instruction on how to assist. With proper restraint, there are no significant risks to the animals involved.

No additional veterinary care will be provided.

After the blood is collected it will be analyzed and the results used in data analysis to compare to the answers to the questionnaire and results from other farms.

These results and any other personal information will not be made available to you personally or any other members of the public. The conclusions drawn from the results will be available and the national and provincial state veterinary services can use it when designing disease prevention and control strategies and policies. Other researchers can also use these results as a reference when conducting further studies into this disease in South Africa.

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

• NAME OF OWNER: _____

FARM NAME/ ADDRESS:_____

• HAVE YOU RECEIVED DETAILED INFORMATION REGARDING THE PROPOSED STUDY? Y/N

- HAVE ALL THE RISKS INVOLVED IN THE PROCEDURE BEEN EXPLAINED TO YOU AND DO YOU FULLY UNDERSTAND THESE RISKS? Y/N
- DO YOU GRANT FULL CONSENT FOR THE PROCEDURE TO BE PERFORMED? Y/N

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

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

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

_____ _____

SIGNATURE RESEARCHER(S) SIGNATURE OWNER

_____

SIGNATURE WITNESS

DATE: _____

Appendix C - Animal Ethics Certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Seroprevalence and Risk Factors of Rift Valley Fever in Cattle and Goats in Free State and Northern Cape Provinces**
PROJECT NUMBER	**V090-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**YB Ngoshe**

STUDENT NUMBER (where applicable)	**UP_14437831**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Cattle**	**Goats**
NUMBER OF ANIMALS	**510**	**510**
Approval period to use animals for research/testing purposes		**October 2015-October 2016**
SUPERVISOR	**Prof. PN Thompson**	

KINDLY NOTE:

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

APPROVED	Date .28 September 2015
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15

Appendix D - Section 20 Approval

agriculture,
forestry & fisheries

Department
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

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

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

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

Dear Prof Paweska

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

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

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

Conditions:

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

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

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

Kind regards,

DR. MPHO MAJA

DIRECTOR OF ANIMAL HEALTH

Date: 2015 -09- 07

SUBJECT: Understanding Rift Valley in Republic of South Africa- Study 1. Ruminant baseline serosurvey.