Rift Valley fever virus circulation in livestock and wildlife, and population dynamics of potential vectors, in northern KwaZulu-Natal, South Africa

by

CARIEN VAN DEN BERGH

Submitted in partial fulfilment of the requirements for the degree

Doctor of Philosophy

in the Department of Veterinary Tropical Diseases,
Faculty of Veterinary Science,
University of Pretoria

Promoter: Prof EH Venter
Co-promoter: Prof PN Thompson
Co-promoter: Prof R Swanepoel

August 2019

DECLARATION

I, Carien van den Bergh, student number 28215461 hereby declare that this dissertation, "*Rift Valley fever virus circulation in livestock and wildlife, and population dynamics of potential vectors, in northern KwaZulu-Natal, South Africa.*", submitted in accordance with the requirements for the Doctor of Philosophy (Veterinary Science) degree at University of Pretoria, is my own original work and has not previously been submitted to any other institution of higher learning. All sources cited or quoted in this research paper are indicated and acknowledged with a comprehensive list of references.

.....

Carien van den Bergh

August 2019

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following people:

- My supervisors, Prof Estelle Venter, Prof Peter Thompson and Prof Bob Swanepoel for their guidance and support.
- Prof Peter Thompson, Ginette Thompson, Dannet Geldenhuys, Yusuf Ngoshe and Bruce Hay for accompanying me to Ndumo for sample collections.
- Prof Paulo Almeida and Dr Louwtjie Snyman, for their assistance with the mosquito identification.
- Ms Karen Ebersohn for assisting me with laboratory work.
- I would like to acknowledge the kindness and patience of the farmers and herders in the study area, as well as the generous assistance of the State Veterinarian and the Animal Health Technicians of the KwaZulu-Natal Department of Agriculture and Rural Development, Jozini District, and Ezemvelo KZN Wildlife.
- We thank Ezemvelo KZN Wildlife for supplying the weather data
- Centers for Disease Control and Prevention, Meat Industry Trust, Cape Wools South Africa and AgriSETA for the funds to do the project.
- Francois van Niekerk, my husband and my mother Ina Muller for their help and support.

The results of this research have been published and reported at conferences:

- Van den Bergh C., Venter E.H.; Swanepoel R., Thompson P.N (2019). High seroconversion rate to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal, South Africa, in the absence of reported outbreaks. PLOS Neglected Tropical Diseases 13(5): e0007296. https://doi.org/10.1371/journal.pntd.0007296
- Poster for Faculty day
- Delegate at Southern African Society for Veterinary Epidemiology and Preventative Medicine
- Delegate at the 15th International Symposium of Veterinary Epidemiology and Economics

ABBREVIATIONS

AIC	Akaike information criterion
Ae.	*Aedes*
An.	*Anopheles*
BHK21	Baby hamster kidney 21
CHIKV	Chikungunya virus
CI	Confidence interval
CPE	Cytopathic effects
Cx.	*Culex*
ELISA	Enzyme-linked immunosorbent assay
FMD	Foot and mouth disease
IEP	Inter-epidemic period
IgG	Immunoglobulin G
IgM	Immunoglobulin M
KNP	Kruger National Park
KZN	KwaZulu-Natal
L	Large
M	Medium
MIR	Maximum Infection Rate
MP12	Rift Valley fever MP12 vaccine strain
N	Nucleocapsid
NGR	Ndumo Game reserve
NSm	Non-structural protein coded by the M-segment
NSs	Non-structural protein coded by the S-segment
OBP	Onderstepoort Biological Products
OD	Optical density
OIE	World Organisation for Animal Health
OR	Odds Ratio
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PP	Positive percentage
qRT-PCR	Real-Time Quantitative reverse transcriptase PCR

RdRp	RNA dependant RNA polymerase
RK13	Rabbit kidney 13
RNP	Ribonucleocapsid
rNP	Recombinant nucleoprotein
RT-PCR	Reverse transcriptase polymerase chain reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
S	Small
SA	South Africa
spp.	Species
SINV	Sindbis virus
SNT	Serum neutralizing test
TCID	Tissue culture infective dose
TEP	Tembe Elephant Park

TABLE OF CONTENTS

Annexure

List of Tables

List of Figures

SUMMARY

Rift Valley fever virus circulation in livestock and wildlife, and population dynamics of potential vectors, in northern KwaZulu-Natal, South Africa

by

Carien van den Bergh

Promoter: Prof E.H. Venter
Professor Emeritus:
University of Pretoria
College of Public Health
Medical and Veterinary Sciences
James Cook University, Australia

Co-promoter: Prof P.N. Thompson
Department of Production Animal Studies
Faculty of Veterinary Science
University of Pretoria

Co-promoter: Prof R. Swanepoel
Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
University of Pretoria

For the degree: Doctor of Philosophy
Department: Veterinary Tropical diseases

Rift Valley fever virus (RVFV) is a mosquito-borne virus and a member of the family *Phenuiviridae* and genus *Phlebovirus.* The single stranded RNA genome consists of three segments, Large (L), Medium (M) and Small (S). Rift Valley fever (RVF) is a mosquito-borne zoonotic disease that may cause large epidemics in ruminants and humans. Infection in humans causes influenza-like symptoms but the disease can also be more severe and fatal. Outbreaks in livestock are classified by abortion storms and young and newborn animals are severely affected with a high mortality rate. Rift Valley fever causes severe health and economic consequences in the areas where it occurs. Since the first recorded incidence of RVF in Kenya in 1930, South Africa has had three major countrywide outbreaks: in 1950-1951, 1973-1975 and 2008-2011. The disease is characterized in southern Africa by large epidemics at long, irregular intervals. The epidemics are usually associated with conditions favourable for proliferation of mosquito populations, such as high rainfall and flooding. Rift Valley fever has previously been isolated from 12 different mosquito species in South Africa including 5 *Aedes* spp., 3 *Culex* spp., 3 *Anopheles* spp. and 1 *Eretmapodites* sp. The presence of the virus and patterns of occurrence of the disease in the eastern parts of South Africa are poorly understood.

Multiple studies were conducted; the aim of the first study was to detect the presence of RVFV in far northern KwaZulu-Natal Province, South Africa and to estimate the incidence rate of seroconversion. Cross-sectional studies were performed in communally farmed cattle (n=423) and goats (n=104), followed by longitudinal follow-up of seronegative livestock (n=253) 14 times over 24 months, representing 160.3 animal-years at risk. Exposure to RVFV was assessed using an IgG sandwich ELISA and a serum neutralization test (SNT) and seroconversion was assessed using SNT. Initial overall seroprevalence was 34.0% (95%CI: 29.5-38.8%) in cattle and 31.7% (95%CI: 22.9-41.6%) in goats, varying by locality from 18-54%. Overall seroconversion rate in cattle was 0.59 per animal-year (95% CI: 0.46-0.75) and in goats 0.41 per animal-year (95% CI: 0.25-0.64), varying significantly over short distances. The high seroprevalence in all age groups and evidence of year-round viral circulation provide evidence for a hyperendemic situation in the study area.

The second study investigated the seroprevalence and associated risk factors of RVFV in antelope in the Tembe Elephant Park (TEP) and the Ndumo Game Reserve (NGR), using 326 sera from nyala (*Tragelaphus angasii*) and impala (*Aepyceros melampus*) routinely culled over a two-year period. The overall seroprevalence of RVFV was 35.0% (114/326; 95% CI 29.8-40.4%); the presence of antibodies in juveniles (6/21; 28.6%; 95% CI 11.3-52.2%) and sub-adults (13/65; 20.0%; 95% CI 11.1-37.8%) confirmed that infections had occurred subsequent to the 2008-2011 RVF outbreaks in South Africa. Seroprevalence was highest in adults and inversely associated with distance from a swamp or floodplain.

The third study aimed to investigate the diversity, abundance, and seasonal dynamics of mosquitoes in the study area, and to screen mosquitoes for RVFV. Monthly collections of adult mosquitoes were carried out from January 2017 to June 2018 at three sites using CO_2-baited tent traps. Mosquitoes were identified, pooled and screened for RVFV by quantitative reverse transcriptase (RT)-polymerase chain reaction (PCR) directed toward amplification of a 217-bp fragment of the L segment. A total of 34,848 mosquitoes of 7 genera and 48 species, were captured; *Culex* (*Cux.*) *tritaeniorhynchus* (31%), *Cx.* (*Cux.*) *antennatus* (29%), *Aedes* (*Adm.*) *durbanensis* (12%) and *Cx.* (*Cux.*) *neavei* (10%) were the most abundant species collected. Genera differences were noted between the collection sites. Cumulative rainfall and average minimum temperatures 30 days prior to collection were positively associated with the number of mosquitoes collected while maximum temperatures were only associated with the number of *Culex* mosquitoes caught.

A single pool of *Ae. durbanensis* was found to be positive for RVFV genomic RNA. The same pool was also positive for Chikungunya virus (Family *Togaviridae*, genus *Alphavirus*) (CHIKV) and Sindbis virus (Family *Togaviridae*, genus *Alphavirus*) (SINV). The RVFV isolate was closely related to one obtained from *Ae. (Neo.) circumluteolus* at Simbu pan in 1955, ±20 km from the collection sites for this study. Further investigation should be done on the human health implications of the presence of these three zoonotic arboviruses. It is possible that these viruses are causing disease among the communities in the area and that the diseases are under-reported.

The results of this study show that RVFV is circulating in the area in domestic ruminants and wildlife, in the absence of apparent clinical disease, at a rate that varies by location, season and year. It appears that, under similar ecological conditions, domestic and wild ruminants may play a similar role in maintenance of viral circulation, and either or both may serve as the mammalian host in a vector-host maintenance system. The study also demonstrates the presence of a wide variety of mosquito species, several of which are known to be competent RVFV vectors.

Chapter 1

1.1 INTRODUCTION

Rift Valley fever (RVF) is an acute arthropod-borne viral disease caused by RVF virus (RVFV), a single-stranded RNA virus in the family *Phenuiviridae* (Adams *et al*., 2017)*;* it is a zoonotic virus that mainly infects ruminants and humans (Pepin *et al*., 2010). Due to the severity of the disease and its capability to cause major epidemics in livestock and humans, RVF has severe health and economic consequences in areas where it occurs (Flick and Bouloy, 2005). Consequently, it has been designated a notifiable disease according to the European Council Directive 82/894/EEC of 21 December 1982 (http://www.vet.gov.ba/pdffiles/eu_leg/anheu19.pdf). The virus is also considered a potential biological weapon in the European Union and the United States of America (Rolin *et al*., 2013).

Rift Valley fever virus was first isolated in Kenya in 1930, although RVF-like disease was reported almost 20 years before in the same region (Daubney *et al*., 1931). The presence of the virus was subsequently recognized in many countries in sub-Saharan Africa, and several major outbreaks occurred in eastern and southern Africa (Bird *et al*., 2009). Occurrence of outbreaks steadily expanded to the northern and western parts of Africa (Fontenille *et al*., 1998), the Arabian Peninsula (Balkhy and Memish, 2003), Madagascar (Andriamandimby *et al*., 2010) and Mayotte (Pepin *et al*., 2010).

Since the discovery of RVFV, South Africa has had three major outbreaks in 1950-1951, 1973-1976 and 2008-2011. The outbreaks were interspersed with extended periods of absence of overt outbreaks (Alexander, 1951, Gear *et al*., 1951, Coetzer, 1977, Barnard, 1979, Perez *et al*., 2010, Archer *et al*., 2011). Human infections were reported among high risk groups including farmers and veterinarians, with infection mainly due to contact with infected animals or infected animal tissues (Archer *et al*., 2011).

Recent research on RVF has focused on the understanding of the aetiological agent and development of diagnostic tests. However, there are still many aspects of the

epidemiology, especially regarding the vectors of RVFV, that are not fully understood. The impact of movement of animals, illegally or by trade, on the spread of RVFV, the possible reservoirs of the virus during inter-epidemic periods (IEPs), and the factors and mechanisms influencing the spread of the virus after the IEP, require further research.

The aim of the present project was to gain insight into the epidemiological dynamics of RVFV in a suspected endemic area in South Africa. Specific objectives included the determination of recent circulation of the virus in the sub-tropical region of far northern KwaZulu-Natal (KZN), South Africa, and estimation of the seroprevalence in cattle, goats and wild ungulates, including nyala (*Tragelaphus angasii*) and impala (*Aepyceros melampus*). The rate of seroconversion was determined in domestic ruminants over a two-year period and a mosquito survey was done in parallel to determine the population and infection status of potential vectors in the area.

1.2 AETIOLOGICAL AGENT

1.2.1 Structure

Rift Valley fever virus is an arthropod–borne virus and a member of the family *Phenuiviridae* and genus *Phlebovirus (*Adams *et al.,* 2017*)*. The virus is spherical, enveloped with a diameter of 80 – 120 nm. The virus capsid consists of glycoprotein spikes composed of glycoproteins N and C (Gn and Gc) projecting through a bi-layered lipid envelope. The glycoproteins form the envelope around the ribonucleocapsid. The single-stranded RNA genome is divided into three segments, large (L), medium (M) and small (S), each with its own nucleocapsid protein (Fig. 1.1). The RNA segments are coated with nucleocapsid (N) protein and are further associated with a few copies of protein L (transcriptase) (Siam *et al*., 1980, Gerdes, 2004, Ikegami *et al*., 2007, Grolla *et al*., 2012).

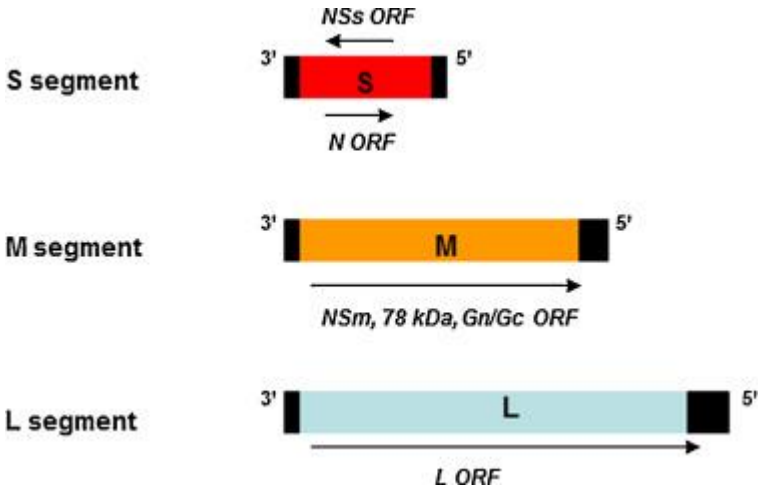


Figure 1.1: Schematic representation of the three single stranded RNA segments of RVFV detailing the small (S), medium (M) and large (L) segments in linear presentation. The black areas of the illustration denote 3' and 5' ends of each segment. (Mansfield *et al*., 2015). https://doi.org/10.1016/j.vaccine.2015.08.020

The L- segment has a negative sense and encodes the 240 kDa L-protein, the RNA-dependent RNA polymerase (RdRp), which is packed together with the genomic RNA segments within the virus particles. This protein is necessary for viral replication *(*Müller *et al*., 1995: Ikegami et al., 2005).

The negative M segment codes for the glycoprotein precursors (Won *et al*., 2007) and non-structural protein (NSm) that is not necessary for replication in mammalian or mosquito cells (Bird *et al*., 2007a, Bird *et al*., 2009, Kakach *et al*., 1988) and has been demonstrated to function in supressing apoptosis (Spik *et al*., 2006, Won *et al*., 2007).

The S-segment encodes the N protein plus a non-structural protein (NSs) and uses an ambisense strategy. The nucleocapsid (N) is translated from the viral sense mRNA (798 nt, 265 amino acids, 34 kDa) while the NSs nucleocapsid (N) (738 nt, 265 amino acids, 25 kDa) is expressed from the anti-sense (Schmaljohn and Nichol, 2007). It has been demonstrated *in vitro* that the NSs protein acts to suppress the induction of the antiviral type I interferon after infection (Billecocq *et al*., 2004). The N protein is required for encapsulation of the viral RNA to form the ribonucleocapsid (RNP) (Ikegami *et al*., 2005).

The RVFV genome is genetically stable as has been shown through decades (Grobbelaar *et al*., 2011). Sequence data and phylogeny indicate that the virus can be grouped into multiple viral lineages (A to O) and viral reassortment occurs infrequently where an isolate contains genome segments originating from different lineages (Ikegami, 2012).

Grobbelaar and co-workers (2011) analyzed 198 isolates of the virus which circulated in affected countries over 67 years. Partial sequencing of the M segment RNA produced 95 unique sequences that resolved into 15 lineages. Lineage A consists of isolates from Zimbabwe (1978), Madagascar (1979), and Egypt (1977-78 and 1993). Lineage C was isolated from major outbreaks in Zimbabwe (1978), Madagascar (1991) and eastern Africa that included Kenya, Tanzania, and Somalia (1997-1998). Viruses isolated during the 2008 and 2009 in South Africa belonged to Lineage C, whereas viruses or strains isolated from the outbreak in 2010-2011 belong to Lineage H. Lineage E comprises isolates from Cameroon/Central African Republic, Zimbabwe (1973-1978) and Zambia (1985). Lineage G viruses comprises isolates from Cameroon/ZAR, Zimbabwe, Guinea and Senegal (1969-1986) (Grobbelaar *et al*., 2011).

No topotypes or viral lineages are bound to a specific geographical area. However, studies have shown that viruses from multiple lineages can circulate in an area during an outbreak (Grobbelaar *et al*., 2011).

1.2.2 Pathogenesis

Rift Valley fever virus binds to an unidentified cellular receptor and enters the cell. It is hypothesized that the virus uses clathrin- mediated endocytic pathways (Lozach *et al*., 2010). Viral uncoating takes place and the ribonucleocapsid is released into the cytoplasm. The viral polymerase that is attached to the ribonucleocapsid exerts primary transcriptase to synthesize viral mRNA. Both N and NSs are transcribed during the primary transcription 40 min after infection of a host cell (Ikegami *et al*., 2005). Viral replication starts within 1 – 2 hours after infection of the animal. Rift Valley

fever virus is transported from the inoculation site to the regional lymph nodes via lymphatic drainage (Ikegami *et al*., 2005, Ikegami and Makino, 2011). The virus may replicate in the lymph nodes and spread from here to initiate primary viraemia; the virus can now infect other organs such as the liver, spleen and the brain. Replication takes place in the hepatocytes of the liver, in the walls of small vessels (adrenocortical cell and glomeruli of the kidney) (Flick and Bouloy, 2005, Grolla *et al*., 2012). Viral antigens can be found in the visceral organs, the brain, and often the fresh placenta of the aborted foetus, with high titres of virus in the serosanguinous fluid found in the thoracic cavity of the foetus especially when abortion took place in late gestation (Flick and Bouloy, 2005).

The virus can be detected in the blood of lambs as early as 16 hours post infection, while death occurs between 35-42 hours post infection (Olaleye *et al*., 1996). In adult ruminants the virus can be detected in the blood after 24 to 48 hours post infection. Viraemia reaches its peak after 2-5 days; the duration of the fatal illness may be 36 to 42 hours (Easterday, 1965); however, the virus can persist in organs such as the spleen and other visceral organs for up to 21 days (Flick and Bouloy, 2005). The virus causes hepatic necrosis, nephrosis, vasculitis, lymphoid necrosis and haemorrhagic disease in adult ruminants (Ikegami and Makino, 2011).

1.2.3 Clinical signs

Rift Valley fever virus infection causes influenza-like symptoms that are self-limiting, in humans, but disease presentation can be more severe, with 8% of RVFV infected patients developing severe complications which may include neurological disorders such as vision loss, haemorrhagic fever, thrombosis and death (Njenga *et al*., 2009). The incubation period is 2-7 days. The onset of the disease is usually marked by rigors, malaise, weakness, dizziness, and severe headaches accompanied by pyrexia (38.8 to 39.5°C), low blood pressure, back pain and red painful eyes. The fever normally breaks on day 4 but some patients experience fever for up to 10 days (Ikegami and Makino, 2011, Elwan *et al*., 1997). The virus can be found in the blood during the febrile period, while antibodies can be found from the fourth day after the onset of illness (Ikegami and Makino, 2011).

Clinical signs in ruminants include pyrexia (39°C to 40°C), anorexia, abdominal pain, general weakness, haemorrhagic diarrhoea, nasal discharge (which in small ruminants is often tinged with blood) and hyperpnoea. Abortions can occur at any stage of pregnancy (Coetzer, 1982).

Domestic ruminants are susceptible to RVFV, but the clinical signs vary and can be non-specific. Adult animals seem to be less severely affected by the disease and the clinical signs can vary from in apparent to per-acute or acute infection (Coackley *et al.,* 1967, Coetzer, 1982). Infected animals exhibit fever, anorexia, nasal discharge, bloody diarrhoea and can also be icteric in some cases. Outbreaks are characterised by abortion storms, and young and newborn animals may be severely affected with a high mortality rate. The mortality rate in sheep and goats is higher than that of cattle (Coetzer and Barnard, 1977, Gerdes, 2004). Very little research has been done with regard to the effect on wildlife, but abortions among different species of wildlife have been recorded. In 1999 abortions in a waterbuck (*Kobus ellipsiprymnus*) and six African buffaloes (*Syncerus caffer*) were confirmed to be caused by RVFV (Olive *et al*., 2012). Unspecified clinical disease, including death, due to RVFV was reported in African buffalo, springbok (*Antidorcas marsupialis*), kudu (*Tragelaphus strepsiceros*), nyala (*T. angasii*), sable (*Hippotragus niger*), roan (*H. equinus*), gemsbok (*Oryx gazella*), blesbok (*Damaliscus dorcas dorcas*), bontebok (*D. dorcas phillipsi*) and waterbuck during the 2010-2011 RVF outbreaks in the interior of South Africa, although in extremely low numbers compared to the large numbers of domestic ruminants affected (Pienaar and Thompson, 2013).

1.2.4 Immune response

The N protein appears to be the major immunogen, but antibodies are also produced against the surface glycoproteins (Suzich and Collett, 1988, Collett *et al*., 1985). Neutralizing antibodies have a protective affect against virulent RVFV and for this reason the presence of antibodies is a good indication of immunization when using a RVFV live attenuated vaccine (Flick and Bouloy, 2005). Rift Valley fever virus induces both adaptive and innate immune responses, which contribute to the clearance of

RVFV in infected animals (Bird *et al*., 2009) Three to five days after the onset of clinical symptoms IgM antibodies starts to appear. This is the end of the viraemic period, and the virus cannot thereafter be detected in the blood. IgM antibodies can be detected for between one and two months, although four months have been reported in some animals. Ten to fourteen days after infection IgG antibodies will appear and can persist for one to two years after infection (ElHassan, 2006). Immune ruminant mothers can pass acquired immunity to offspring which will protect the offspring for the first three to four months of their lives (Smithburn, 1949).

1.2.5 Control

Due to the economic importance of the disease, a veterinary vaccine was developed (Smithburn, 1949). The Smithburn neurotropic strain was used to produce the first vaccine in 1944. This was done by intracerebral passages of the Entebbe strain in suckling mice (Smithburn, 1949, Caplen *et al*., 1985) but it was not successful since the strain provoked a range of anomalies of the central nervous system in foetuses such as porencephaly, hydraencephaly and microencephaly. The Smithburn neuro-adapted virus was obtained by Onderstepoort Veterinary Institute South Africa in 1951 (Alexander, 1951) and passaged further in mice and embryonated duck eggs and issued as a vaccine in 1951 (Kaschula, 1953). Vaccinating ewes resulted in stillbirths and abortion. Subsequently reversion was made to mouse-passaged virus and several adjustments in passage level were made, and from 1971 the virus has been grown in BHK21 cells for preparation of freeze-dried vaccine (Swanepoel & Coetzer 2004). The Smithburn strain is used in South Africa for cattle, sheep and goats® (Reg. No. G0119 (Act 36/1947) and is used to vaccinate ruminants annually in South Africa

A formalin inactivated RVFV vaccine (Reg. No. G1349 (Act 36/1947) is available from Onderstepoort Biological Products (OBP); the vaccine requires a booster 3-4 weeks after the initial dose is given and it is recommended that vaccination is given annually. (https://www.obpvaccines.co.za/Cms_Data/Contents/OBPDB/Folders/Product/~contents/GZC2QWWS33BNW7VE/2146%20Inactivated%20RVF_PI.pdf)

The mutagenized strain MP12, was derived from virus isolated in Egypt in 1977 (Caplen *et al*., 1985). Experimental inoculation of ruminants showed efficient immune reaction to the strain (Morrill *et al*., 1997a, Morrill *et al*., 1997b), but MP12 was still teratogenic and caused abortions in ewes during the first trimester of pregnancy (Morrill and Peters, 2003).

A virus originally isolated from a human case, with a large deletion in the gene coding for the non-structural protein NSs, is used in the Clone 13 vaccine and it produces efficient immunity in mice (Vialat *et al*., 2000). The deletion makes reversion to virulence unlikely and is therefore ideal to use in endemic situations (Muller *et al*., 1995). The live attenuated RVF Clone 13® vaccine (Reg. No. G3876 (Act 36/1947) Namibia: NSR 1516) produced by OBP was tested and is available in South Africa as an annual single injection vaccine for cattle sheep and goats (Ikegami and Makino, 2009).

Since the disease disappears for long intervals of 10 – 15 years (see discussion in Section 1.3.4), farmers in South Africa do not in general vaccinate domestic ruminants as recommended. Vaccination normally occurs at the onset of an outbreak as a desperate attempt to prevent further spread of the disease (Gerdes, 2004).

A safe and effective formalin inactivated RVFV vaccine, originally developed for USA soldiers serving in the Middle East as well as to protect laboratory staff was used in humans (Randall *et al*., 1962). It is, however, no longer available. No other vaccines for human use are currently available (Ikegami and Makino, 2009).

1.2.6 DIAGNOSIS

Initial recognition of RVFV is mainly based on observing abortion storms in goats, sheep and cattle and fatalities among new-born animals after the occurrence of abnormally heavy rain. This is usually accompanied by febrile disease in livestock workers and veterinarians (Swanepoel and Coetzer, 2004).

Many different procedures have been published for the detection of RVFV in human and animal samples as well as vectors. The virus can be isolated from aborted foetal tissue, blood, liver and mosquito vectors using primary or secondary cell lines, as well as laboratory animals such as suckling mice (Shimshony and Barzilai, 1983, Swanepoel *et al*., 1986). The following cell lines have been used to isolate the virus: mammalian cell cultures, obtained from tissue of bovine, porcine, monkey and hamster origin, such as Vero, baby hamster kidney (BHK-21) and rabbit kidney (RK13) cell lines. The virus produces clear cytopathic effect (CPE) in mammalian cell lines and the CPE is most distinct in BHK-21 cells (Miura *et al*., 1991). Viral isolation is the diagnostic test of choice to confirm the identity of the virus according to World Organisation for Animal Health (OIE) standard (OIE, 2009). Mice inoculated with RVFV-infected material via the intracerebral route will die in 2 – 5 days post-infection (Swanepoel, 1981). Histopathology and immunohistochemistry can be used to diagnose RVF in organs such as the liver. The use of histopathology in in combination with immunohistochemistry on formalin-fixed samples constitutes a convenient diagnostic method that overcomes problems in preserving specimens during transport to the laboratory (Odendaal *et al*., 2014).

Molecular assays have been developed that use the reverse transcriptase-polymerase chain reaction (RT-PCR) (Ibrahim *et al*., 1997, Jupp *et al*., 2000); nested PCR (Ibrahim *et al*., 1997, Sall *et al*., 2002) and quantitative reverse transcriptase (real-time) PCR (qRT-PCR) (Garcia *et al*., 2001, Drosten *et al*., 2002, Bird *et al*., 2007b). Most of these assays use primers that are designed to detect the G2 glycoprotein gene of the M-segment, but primers have also been based on conserved regions of the S and L segments. In the Mauritania outbreak in 1998 a nested RVFV RT-PCR was used to screen samples from clinically ill patients (Sall *et al*., 2002). A qRT-PCR was successfully used to rapidly diagnose the infection when high fatalities occurred during an outbreak in Kenya (Njenga *et al*., 2009). Real-time loop-mediated isothermal amplification assay was recently developed. This assay detects RVFV nucleic acid in samples within 30 minutes. A major advantage for this test is that it can be done with a portable device (Le Roux *et al*., 2009).

The serum neutralization assay (SNT) and enzyme linked immunosorbent assays (ELISA) are most commonly used as serological assays. SNTs are used to test serum for the presence of neutralizing antibodies in naturally infected or vaccinated animals. The assay is the recommended by the OIE due to early response, specificity and sensitivity (OIE, 2012).

Rift Valley fever virus activity can also be demonstrated with various modifications of ELISAs including antigen detection, IgM and competition or blocking ELISAs (Paweska *et al*., 2003, Paweska *et al*., 2005a, Paweska *et al*., 2005b, Paweska *et al*., 2007, Paweska *et al*., 2008). These assays are widely used in surveillance studies, disease control and monitoring of immunological response after vaccination (Kortekaas *et al*., 2011). The ELISAs have largely replaced established methods including complement fixation, haemagglutination inhibition, agar gel immunodiffusion and plaque-reduction neutralization (Flick and Bouloy, 2005). ELISAs with highly specific monoclonal antibodies have been used for rapid RVFV detection in human sera (Zaki *et al*., 2006) and assays with recombinant proteins have been used in large surveys (Van Vuren *et al*., 2007). A diagnostic antigen recombinant nucleoprotein (rNP) has been used to validate an indirect ELISA (I-ELISA) of RVFV for human, domestic ruminants and African buffalo (Paweska *et al*., 2007, Fafetine *et al*., 2007, Paweska *et al*., 2008). The disadvantage is that the assay can be difficult to develop as it uses monoclonal antibodies that may difficult to produce in smaller laboratories with restricted resources. This assay has been used to detect antibodies in buffaloes in Kenya during an IEP (Evans *et al*., 2008).

1.3 EPIDEMIOLOGY

1.3.1 Hosts

Rift Valley fever is a zoonotic disease and human infections are usually related to specific occupations which place individuals at risk. Farmers, farm and abattoir workers and veterinary personnel who are directly exposed to infected animal tissue are at high risk of contracting the virus (Archer *et al*., 2011).

Wild and domestic ruminants (cattle, goats, sheep and cattle) are the main hosts for RVFV, including African buffaloes and antelope including Thomson's gazelle (*Gazelle thomsonii*), sable (*Hippotragus niger*), impala (*Aepyceros melampus*), kudu, eland (*Taurotragus oryx*), nyala and kongoni (*Alcelaphus buselaphus*), can be infected (Olive *et al*., 2012, Evans *et al*., 2008). Other susceptible hosts include giraffes (*Giraffe cemelopardalis*), African elephant (*Loxodonta Africana*), black rhinoceros (*Diceros bicornis*) camels (*Camelus*), dogs, cats and ferrets (*Mustela putorius furo*) (Daubney *et al*., 1931, Scott *et al*., 1963). The role of wildlife in the epidemiology and ecology of RVFV is still not fully understood (Olive *et al.*, 2012). Wildlife that are not susceptible to infection include lions (*Panthera leo*) and wild equines (*Equus burchelli*) (Evans *et al*., 2008). Furthermore, no symptomatic disease has ever been recorded in horses although in the lower Nile Valley RVFV antibodies were detected during an IEP (Meegan *et al*., 1979, Hoogstraal *et al*., 1979) and in Nigeria antibodies in horses were detected using a complement fixation test (Olaleye *et al*., 1996). Serosurveillance failed to detect RVFV among birds resulting in the exclusion of the class Aves from RVFV studies (Findlay and Daubney, 1931, Findlay, 1932, Gear *et al*., 1951, Gear *et al*., 1955). Some wild animals including amphibia and poultry are resistant to RVFV (Lefèvre, 1989).

1.3.2 Vectors

Mosquitoes (Diptera: Culicidae), particularly culicine mosquitoes, are the principal vectors of RVFV and the virus has been successfully isolated from 53 species of mosquitoes. Transmission of the virus has been linked to the *Aedes*, *Culex*, *Anopheles* and *Mansonia* genera (Meegan and Bailey, 1989). Mechanical transmission under laboratory conditions is also possible by phlebotomid flies (Diptera: Phlebotominae), *Culicoides* midges, stomoxids (Diptera: Muscidae), simulids (*Simuliidae*), and other biting flies and ticks (Hoch *et al*., 1984, Turell and Perkins, 1990).

Aedes (subgenera *Aedimorphus* and *Neomelaniconion*) mosquitoes can transmit RVFV transovarially and lay their eggs in muddy areas. The shallow, standing water of pans and vleis (dambos) are ideal breeding habitats (Linthicum *et al*., 1983, Linthicum *et al*., 1984b, Linthicum *et al*., 1984a, Logan *et al*., 1991). The drought-

resistant eggs can remain viable for long periods of time. With heavy rainfall the eggs are flooded, and large numbers of mosquitoes emerge (Linthicum *et al*., 1985). These infected mosquitoes may spread the virus to animals (Pepin *et al*., 2010). After *Aedes* spp. emerge, they are replaced by large numbers of so-called epidemic vectors, *Culex* mosquitoes (Jupp, 2005), which also lay their eggs in permanent standing water (Jupp, 1996). Adult mosquitoes become infected when they feed on infected animals, and then transmit the virus to other animals (Gad *et al*., 1999).

Rift Valley fever virus has been isolated from 14 mosquito species in South Africa, including 5 *Aedes* spp., 3 *Culex* spp., 3 *Anopheles* spp. and 1 *Eretmapodites* sp. (Swanepoel and Coetzer, 2004). Altogether, RVFV has been isolated from over 53 mosquito species in 8 genera (Linthicum *et al*., 2016). In Africa, 23 mosquito species have been shown to be capable of RVFV transmission under both experimental and field conditions. The 14 species found to be infected in South Africa include: *Eretmapodites quinquevittatus, Culex zombaensis* (McIntosh *et al*., 1973)*, Aedes juppi* (Gargan *et al*., 1988, Jupp and Cornel, 1988, McIntosh *et al*., 1980a)*, Aedes caballus* (Gear *et al*., 1955), *Culex theileri* (McIntosh, 1972, McIntosh *et al*., 1980a)*, Culex neavei* (McIntosh *et al*., 1973)*, Anopheles squamosus* (McIntosh *et al*., 1980a)*, Anopheles cinereus* (McIntosh *et al*., 1980b)*, Aedes mcintoshi* (McIntosh, 1972, Linthicum *et al*., 1985, Abdel Aziz, 2008)*, Aedes dentatus* (McIntosh, 1972)*, Aedes circumluteolus* (Kokernot *et al*., 1957, McIntosh *et al*., 1973, McIntosh *et al*., 1980a, Jupp *et al*., 1984, Turell *et al*., 2008) and *Aedes cinereus* (McIntosh *et al*., 1980a). The mosquito species implicated in outbreaks are listed in Table 1.

The main ecological regions of RVF distribution in South Africa include the temperate inland plateau west of the Drakensberg, and the subtropical lowlands of the eastern seaboard (McIntosh *et al*., 1980a). Outbreaks on the plateau have mostly been reported in cattle and sheep, with associated human cases, during abnormally wet seasons (McIntosh, 1972). In the eastern lowlands sporadic activity of RVFV has been recorded. The infections are mainly observed in cattle, with minimal human infections (McIntosh *et al*., 1980a).

The extrinsic incubation period in the mosquito as well as the competency of the vector to transmit the virus is influenced by ambient temperature and the infective dose (Elliott, 2009). The extrinsic incubation period for RVFV averages 7-14 days and once the female mosquito becomes infected, she remains infected for life. The average mosquito lifespan is about 4 weeks, although infection with the virus may cause inefficient feeding, reduced fecundity and a lower survival rate for mosquito vectors (Dohm *et al*., 1991, Faran *et al*., 1987). Pathogenicity of the virus for mosquitoes increases the transmission rates since inefficient engorgement results in feeding on multiple hosts (Elliott, 2009).

Table 1.1: Mosquito species from which RVFV has been isolated previously.

Species	Location - year	Reference
Aedes aegypti	USAMRIID colony, Sudan 2007	Hoch *et al*., 1985, Seufi and Galal, 2010
Aedes aegypti fomosus	Sub-Saharan Africa, 1983	Jupp *et al*., 1984
Aedes africanus	Uganda 1954, 1956	Weinbren *et al*., 1956
Aedes caballus	South Africa 1953	Gear *et al*., 1955
Aedes cinereus	South Africa 1974, 1975	McIntosh *et al*., 1980a
Aedes circumluteolus	SA 1955, 1956,1981, Uganda 1955	Kokernot *et al*., 1957, McIntosh *et al*., 1973, McIntosh *et al*., 1980a, Jupp *et al*., 1984, Turell *et al*., 2008
Aedes cumminsii	Burkina -Faso 1983	Fontenille *et al*., 1998, Meegan and Bailey, 1989
Aedes dalzieli	Sengal 1974; Kenya 1982	Fontenille *et al*., 1998, Meegan and Bailey, 1989
Aedes dendrophilus	Uganda 1944	Smithburn *et al*., 1948
Aedes dentatus	Rhodsia 1969	McIntosh, 1972
Aedes durbanensis	Kenya 1937	Steyn and Schulz, 1955
Aedes fowleri	Senegal 2002	Ba *et al*., 2012
Aedes furcifer	Burkina -Faso 1983	Fontenille *et al*., 1998, Meegan and Bailey, 1989

Aedes juppi	South Africa 1974, 1975, 1978, 1984	Gargan *et al*., 1988, Jupp and Cornel, 1988, McIntosh *et al*., 1980a
Aedes mcintoshi/lineatopennis	Rhodesia 1969; Kenya, 1983, Kenya1997-8/2006	McIntosh, 1972, Linthicum *et al*., 1985, Abdel Aziz, 2008
Aedes ochraceus	Senegal 1991, Kenya 2006	Fontenille *et al*., 1998, Lutomiah *et al*., 2014, Sang *et al*., 2010
Aedes palpalis	CAR 1969	Digoutte *et al*., 1974
Aedes pembaensis	Kenya 2006	Sang *et al*., 2010
Aedes rufipes	Mauritania 1987,1998 and 2003	Faye *et al*., 2007, Digoutte and Peters, 1989, Nabeth *et al*., 2001
Aedes tarsalis	Uganda 1944	Smithburn *et al*., 1948
Aedes triseriatus	USAMRIID colony	Easterday *et al*., 1962
Aedes vexans arabiensis	Saudi Arabia 2000; Sengal 1987, 2003	Miller *et al*., 2002, Faye *et al*., 2007
Aedes vittatus	Saudia Arabia 2000	Arishi *et al*., 2000
Anopheles christyi	Kenya 1982	Meegan and Bailey, 1989
Anopheles cineres	South Africa 1974, 1975	McIntosh *et al*., 1980b
Anopheles coustani	Rhodesia 1969; Kenya, 1983, Kenya1997-8/2006	McIntosh, 1972, Abdel Aziz, 2008
Anopheles gambiae arabiensis	Sudan 2007	Seufi and Galal, 2010
Anopheles pharoensis	Kenya 1982	Meegan and Shope, 1981
Anopheles squamosus	South Africa 1975; Madagascar 2008; Kenya 2006	McIntosh *et al*., 1980a, Lutomiah *et al*., 2014, Ratovonjato *et al*., 201
Coquillettidia fuscopennata	Kenya 1930; Uganda 1960	Linthicum *et al*., 2016, Williams *et al*., 1960
Coquillettidia grandidieri	Madagascar 1979	Meegan and Bailey, 1989
Coquillettidia microannulata	Kenya 1932	Linthicum *et al*., 2016
Coquillettidia versicolor	Kenya 1932	Linthicum *et al*., 2016

Culex antennatus	Mauritania 1987,1998 and 2003	Digoutte and Peters, 1989, Nabeth *et al*., 2001, Faye *et al*., 200
Culex bitaeniorhyncus	Kenya 2006	Lutomiah *et al*., 2014
Culex decens	Mauritania 1987,1998 and 2003	Digoutte and Peters, 1989, Nabeth *et al*., 2001, Faye *et al*., 2007
Culex fatigans	Kenya 1930	Linthicum *et al*., 2016
Culex neavei	SA, 1973; Mauritania 1987,1998 and 2003	McIntosh *et al*., 1973
Culex pipiens	Egypt 1977, Sudan 2007	El-Akkad, 1978, Meegan *et al*., 1979, Seufi and Galal, 2010
Culex poicilipes	Senegal 1987,2003; Mauritania 1987,1998 and 2003	Digoutte and Peters, 1989, Nabeth *et al*., 2001, Faye *et al*., 2007
Culex quinquefasciatus	Mauritania 1987,1998 and 2003	Digoutte and Peters, 1989, Nabeth *et al*., 2001, Faye *et al*., 2007
Culex simpsoni	Madagascar 2008-09	Ratovonjato *et al*., 2011
Culex theileri	Rhodesia, 1969; Kroonstad & Johannesburg SA, 1970,	McIntosh, 1972, McIntosh *et al*., 1980a
Culex tritaeniorhynchus	Saudi Arabia 2000	Jupp *et al*., 2002
Culex univittatus	Madagascar 2008-09	Ratovonjato *et al*., 2011
Culex vasomereni	Madagascar 2008-09	Ratovonjato *et al*., 2011
Culex zombiensis	South Africa 1973; Kenya 1997-8/2006	McIntosh *et al*., 1973, Abdel 2008
Eratmapodites chrysogaster	Uganda 1944	Smithburn *et al*., 1948
Eratmapodites quinquevittatus	Port Shepstone, SA 1971	McIntosh, 1972, McIntosh *et al*., 1973, Linthicum *et al*., 1985
Eratmapodites rubinotus	Kenya 1982	Meegan and Bailey, 198
Mansonia africana	Kenya 1997-8/2006	Abdel Aziz, 2008
Mansonia uniformis	Kenya 1997-8/200	Abdel Aziz, 2008

1.3.3 Transmission

Biological transmission refers to the infection of a vector in which the virus can multiply to the extent that the virus is transferred when an animal is bitten. The RVF virus is transmitted through the saliva of infected mosquitoes. Although not generally considered important in RVF, another method of transmission is when one infected animal/host infects another mechanically through direct contact with infected blood or body fluids. Mechanical vectors of RVFV may include biting flies such as *Simulium* spp. and *Glossina morsitans*; ticks such as *Amblyomma variegatum* (Fontenille *et al*., 1998), *Hyalomma truncatum* (Linthicum *et al.*, 1989) and *Rhipicephalus appendiculatus* (Daubney and Hudson, 1933); *Culicoides* spp. (Davies and Highton, 1980); sandflies including *Phlebotomus* spp. and *Sergentomyia* (Dohm *et al*., 2000), as well as tapeworm (*Taenia crassicolis*) (Findlay and Howard, 1951). The presence and circulation of the virus is correlated with rainy seasons, which also favour a high population density of mosquitoes. Outbreaks of RVF after long IEPs may be initiated by *Aedes* spp. with the hatching of larvae infected via transovarial transmission of virus (Linthicum *et al*., 1985). Floodwater breeding *Aedes* mosquitoes lay their eggs on the edge of shallow waterlogged depressions. These eggs then require a period of drying out and will then hatch as soon as they are flooded with water during the next rainy season. Not all the eggs will hatch the first time that they are flooded, and it is not known how long the eggs can survive in dry mud or on vegetation, but it has been hypothesised to be months or even years (Linthicum *et al*., 1985). This phenomenon can explain how the virus survives the winter or the long IEPs (Elliott, 2009, Bengis *et al*., 2010). Transovarial transmission allows infected adult mosquito vectors to emerge from breeding habitats after periods of high rainfall to complete their life cycle in 14-21 days. These vectors can spread the virus to any available animals (Watts *et al*., 1973).

Horizontal transmission may occur when the virus is transmitted from animal to animal through licking one another or aborted tissue (Pepin *et al.*, 2010, Pépin, 2011) although not considered to be important in livestock in Africa. The use of contaminated needles to vaccinate multiple animals and artificial insemination with infected semen can also lead to the infection of animals (Eaglesome and Garcia, 1997, Bird and McElroy, 2016).

Humans are exposed to the virus by means of inhalation during the slaughter process of an infected animal (Abu-Elyazeed *et al*., 1996). Contact with bodily fluids of infected animals can also infect humans through abraded skin (Francis and Magill, 1935, Swanepoel and Coetzer, 2004). The disease can be transmitted with the consumption of unpasteurized milk and from mother to child though transplacental infection in pregnant woman (LaBeaud *et al*., 2010, Ikegami and Makino, 2011).

1.3.4 Occurrence

The virus was first isolated from wool sheep and humans in the Rift Valley of Kenya after heavy rains in 1930-1931 (Daubney *et al*., 1931). Since then the disease has reappeared in Kenya at irregular intervals of 3 to 15 years. Another outbreak occurred in 1997-98 in the drier areas of northern Kenya and southwest Somalia that followed heavy rains. The outbreak resulted in human and livestock infections and affected especially camels which disrupted livestock export to the Middle East (FOA, 2003). A recent major outbreak in East Africa was in 2006-07 where high morbidity and mortality rates were recorded in livestock and cattle. The disease has also spread to new regions of the country including Kitui, Tharaka, Meru South, Meru central, Mwingi, Embu, and Mbeere in Eastern Province, Malindi and Taita Taveta in Coast Province, Kirinyaga and Murang'ain Central Province, and Baringo and Samburu in Rift Valley Province (Munyua *et al*., 2010). The most recent outbreaks was reported in Uganda from 2016-18 (Nyakarahuka *et al*., 2019).

Rift Valley fever virus caused a large outbreak in South Africa in 1950 with severe economic losses, when an estimated 100 000 sheep died and at least 500 000 aborted (Gear *et al*., 1951, Pienaar and Thompson, 2013). A second large outbreak followed during 1974-1976, that included both South Africa and Namibia (McIntosh *et al*., 1980a).

In 1967, RVFV was first isolated in Nigeria, West Africa (Meegan and Bailey, 1989). An outbreak of RVFV was then reported in the Kosti District, in the West Nile Province of the Sudan in 1973. The virus was found in almost every locality of the district and spread northwards to the Blue Nile Province and northern part of the White Nile

Province. Humans, sheep, goats and cattle were involved, while mortality in lambs (96%) was the highest followed by goat kids and calves (Eisa *et al*., 1980). Following the outbreak in 1973 in Sudan the virus was introduced into Egypt in 1977, where it was responsible for a severe outbreak between 1977 and 1979 (Meegan, 1979). The results were devastating as 600 human deaths were recorded and there were severe losses of sheep, cattle, goats, buffaloes and camels. The 1977 Egypt outbreak followed building of the Aswan Dam and an increase in irrigation that in turn increase breeding sites for vectors. A second outbreak followed in 1993 and RVFV was isolated from infected calves in Aswan in Egypt in the summer of 1997 (Abd *et al*., 1999).

In 1987, RVFV caused an outbreak in southern Mauritania and northern Senegal that was associated with the closing of the Diama dam on the Senegal River (Balkhy and Memish, 2003, Wilson *et al*., 1994). The disease was known to be endemic in this area but has not caused a high level of morbidity and mortality before (Lederberg, 1993). This was followed with another outbreak in 1998 in the same area. Thirteen isolates of RVFV was isolated during this outbreak and 16% of 90 human samples were IgM positive with one casualty. Animals including sheep, goats, camels, cattle and donkeys were affected during this outbreak (Nabeth *et al*., 2001).

The virus was first detected outside the African continent in Madagascar in 1900-1991 it was suggested that the virus was introduced with importation of animals (Morvan *et al*., 1991). The virus then emerged in the Arabian Peninsula in September 2000 where it caused outbreaks in Yemen and Saudi Arabia. The mechanism of introduction was not confirmed but active livestock trade between Arabian Peninsula and East Africa was suspected (Balkhy and Memish, 2003, Shoemaker *et al*., 2002). The affected area, the Tehama plain, is about 50 km wide, located in the West side of Saudi Arabia, and is located between mountains and the Red Sea. The ecological characteristics of this region are similar to characteristics of the western side of the Rift Valley in Africa. Here the virus activity is highly associated with riverine alluvium zones. The virus emerged because modifications in the ecology increased the amount of permanent fresh water making the environment more favourable for the virus to survive and spread via mosquitoes. During this outbreak major abortion storms among sheep and goats and 884 human cases were reported of which 124 were fatal (Shoemaker *et al*.,

2002). Rift Valley fever virus has been present on the island of Mayotte in the Indian ocean since 2004 but remained endemic since 2011. The serological study was conducted in three phases with the first from 2004-2008 (IgM prevalence = <15%), the second phase during 2008-2010 (IgM prevalence = 41%) the IgM prevalence was similar across all age groups. The last phase was during 2010-2015 (IgM prevalence 10-15%). The study hypothesized that animal imports and rainfall pattern were responsible for the epidemic dynamics (Métras *et al*., 2016). In 2009, there were suspected RVFV circulation which was confirmed during a 2010-2011 longitudinal survey in Grande Comoro, Mohali and Anjouan. The RVFV antibody prevalence between the location; 8.2% in Grande Comoro, 72.3% in Mohali and 5.8% in Anjouan. The infection in Grande Comoro could be correlated with animal trade from the East coast of Africa (Roger *et al*., 2014).

1.3.5 Inter-epidemic period

Outbreaks of RVF in sub-Saharan Africa have always been associated with short periods of high rainfall, although not all wet periods lead to an outbreak (Swanepoel and Coetzer, 2004). Inter-epidemic periods may be as long as 10 - 15 years with no clinical outbreaks reported (Gerdes, 2004). It is not known where the virus survives during this period although multiple hypotheses have been suggested, of which one or more may play a role in different areas:

An initial hypothesis was that the virus circulates in the forest and the vectors breed in holes in trees. High mosquito populations following heavy rains facilitate the spread of the virus to nearby ruminants. The possibility of endemicity in forests cannot be ignored but is most likely not responsible for large outbreaks (Swanepoel and Coetzer, 2004).

Another hypothesis is that the virus persists in *Aedes* mosquito species breeding in shallow grasslands and dambos through transovarial transmission (Linthicum *et al*., 1985). The female mosquitoes lay eggs at the base of vegetation on the edges of accumulated water. Drying of the water results in conditioning of the eggs through partial desiccation. With the next flooding the eggs hatch resulting in high numbers of

transovarially-infected vectors that transmit RVFV to nearby ruminants (Linthicum *et al*., 1985, Davies *et al*., 1985).

Another hypothesis is that the virus circulates continuously at a low level between vectors and mammalian hosts, which may include livestock and wildlife. Evans *et al.* (2008) reported the prevalence of RVFV neutralizing antibodies in 896 samples collected from 16 different wildlife species born in an IEP in Kenya between 1999 and 2006. Species presenting with neutralizing antibody included African buffalo (*Syncerus caffer*), black rhinoceros, kudu (*Tragelaphus strepsiceros*), impala (*Aepyceros melampus*), African elephant (*Loxodonta*), kongoni (*Alcelaphus buselaphus*) and waterbuck. The detection of low-level viral activity in endemic areas is difficult but may be possible if continuous surveillance is conducted. Virus can also be detected by random isolation of the virus from mosquitoes (ElHassan, 2006).

The fourth hypothesis is the possibility that the virus can spread over long distances via infected mosquitoes carried by wind. Due to climatic conditions constantly changing, vectors can become established in areas previously free from the disease (Metras *et al*., 2012).

The last and most plausible hypothesis is movement of infected animals. Both trade and illegal transboundary movement-of infected animals contribute to the distribution of the disease (Balkhy and Memish, 2003). Infected wild and domestic ruminants can function as amplifying hosts, propagating transmission of the virus to the vectors in the areas of introduction (Torres-Vélez and Brown, 2004). It is postulated that the virus was introduced into Arabian Peninsula (Shoemaker *et al*., 2002) with the importation of infected livestock from the east of Africa. The isolates from this outbreak were similar to the 1997-1998 outbreak in East Africa. It was suggested that virus had been circulating at low levels from the time of possible introduction in 1997-98 until the conditions favoured the outbreak of the disease in 2000 (Madani *et al*., 2003, Bird *et al*., 2009). Similar RVFV introductions through importation of livestock has been observed in Madagascar (Jeanmaire *et al*., 2011, Carroll *et al*., 2011) and Comoros (Carroll *et al*., 2011, Cêtre-Sossah *et al*., 2012).

Include a small section on risk factors for the disease in livestock and humans – age, sex in animals; age, sex, occupation in humans

1.3.6 Rift Valley fever in South Africa

South Africa has experienced three major outbreaks to date (Pienaar and Thompson, 2013). The first outbreak was in 1950-1951, when the disease started in the western Orange Free State (Free State Province) and spread towards the northern Cape Province and southern Transvaal (Gauteng Province) (Alexander, 1951). The disease was recognized when a veterinarian became ill after he examined and opened an infected carcass (Mundel and Gear, 1951). During this outbreak both sheep and cattle were affected (Gear *et al*., 1951). Another 28 lesser outbreaks were reported during 1955-1956 in the Orange Free State (Grobbelaar *et al*., 2011). No further outbreaks were reported until 1969-1970. During the IEP, several small outbreaks were reported in Standerton (Mpumalanga), Frankfort, Kroonstad, Koppies, OdendaaIsrus (all Free State Province), Vryburg (North West Province), and the Lower Umfolozi district (KZN Province).

An outbreak caused by viruses from Lineage L (Grobbelaar *et al*., 2011) started in 1974-1976 when areas including the Western Cape, the southern Free State and western parts of North West Province were severely affected, with mortalities as high as 95% occurring among young lambs (Division of Veterinary Services 1974). Thereafter, several cases were reported during the IEP in the KZN and Western Cape provinces and the eastern part of Northern Cape provinces in 1983 -1998 (Division of Veterinary Services 1974). In 1985-1986, 13 more small outbreaks were reported around Estcourt, Utrecht and Ubombo areas of KZN and in 1990-1991, 5 more outbreaks occurred in the Estcourt and Klipivier districts in KZN (Pienaar and Thompson, 2013).

Lineage C RVFV (Grobbelaar *et al*., 2011) recently emerged in South Africa with severe economic, social and health effects. Between 2008 and 2009, both South Africa and Madagascar had RVF epidemics with Madagascar alone reporting 476 human cases including 19 deaths. High fatalities in cattle were also reported

(Andriamandimby *et al*., 2010). In 2008 South Africa reported a total of 8 human cases, mainly among farmers and veterinarians, due to contact with infected animals or infected animal tissue (Archer *et al*., 2011). In 2010, South Africa experienced an almost countrywide outbreak caused by Lineage H, but mainly involving the Free State and the Northern, Eastern and Western Cape Provinces, recording approximately 9000 deaths among livestock, and 200 human cases with 25 deaths (Pienaar and Thompson, 2010). In 2011, only South Africa in the region reported occurrence of the disease (Metras *et al*., 2012). The distribution of the outbreaks during 2008-2011 is displayed in Fig.1.2.

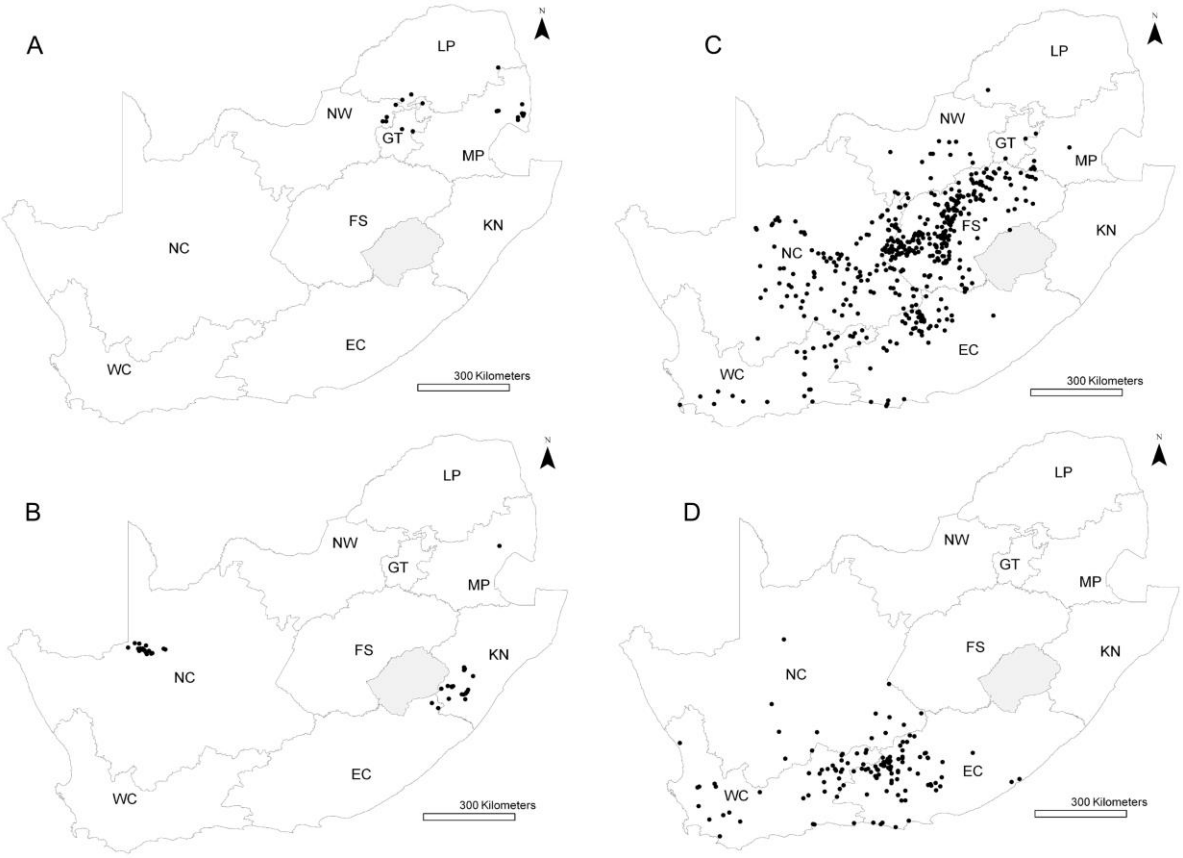


Figure 1.2: Livestock cases of Rift Valley fever in South Africa for 2008 (A), 2009 (B), 2010(C) and 2011 (D). For 2009, both outbreaks are displayed. Provinces are NC: Northern Cape, WC: Western Cape, EC: Eastern Cape, FS: Free State, NW: North West, KN: KwaZulu-Natal, MP: Mpumalanga, GT: Gauteng, LP: Limpopo. The light grey shaded area is Lesotho (no data). Adapted from (Metras *et al*., 2012).

Historically therefore, large outbreaks in South Africa occur on the interior plateau of the country; however, evidence of the presence of RVFV and smaller outbreaks also occur in the east of the country. In 1955, RVFV was isolated from *Ae. circumluteolus* that was caught next to the Simbu pan in KZN (Kokernot *et al*., 1957) and at the same time neutralizing antibodies to RVFV were found in 12% of domestic livestock (Kokernot *et al*., 1961). There have not been any reports of clinical cases of RVF in that region, although further south along the KZN coast there is some evidence of RVFV circulation and sporadic clinical cases in 1972-1973 (McIntosh *et al*., 1980). During a small outbreak on a dairy farm on the coast of Natal, it was reported that 7 isolations from *Cx. zombaensis* and one isolation from *Cx. neavei* were made (Jupp,

1983, McIntosh, 1983). Very few outbreaks have been reported in neighboring Mozambique. Serological evidence in 2010-11 showed that RVFV-specific antibodies are present in sheep and goats without clinical signs (Fafetine *et al*., 2013) and a small outbreak among goats was reported in the Maputo Province in 2014 (Fafetine *et al*., 2016). The overall seroprevalence of RVFV antibodies in seven provinces of Mozambique was reported to be 37% in cattle, 30% in African buffalo, and 29% in domestic ruminants in the Maputo Province, close to the South African border (Moiane *et al*., 2017). Very little is known about the status of RVFV in the north-eastern sub-tropical and tropical parts of South Africa, but it is suspected that there might be low level endemic circulation of RVFV.

1.4 STUDY AREA

Far northern KZN, at the southern end of the Mozambique coastal plain, includes a wide diversity of aquatic and wetland habitat units and includes various aquatic ecoregions. The aquatic and wetland habitats support a great diversity of faunal and floral species. This is due to the area incorporating both subtropical and temperate features that are governed by the warmer Mozambique and Agulhas Ocean currents, and that converge with the northern influence of the relatively cooler Benguela current (Lutjeharms and Roberts, 1988). The northern part of the area is a foot and mouth disease (FMD) controlled zone. Cattle are dipped periodically against ectoparasites and inspected by veterinary authorities for diseases at dip tanks. The nine dip tanks selected for the study area are indicated in Fig. 3, together with various wetlands and rivers. The area is rich in wildlife, as Mkuze Game Reserve, Ndumo Game Reserve and Tembe Elephant Park border the dip tanks in the area. Livestock farming mostly involves communal grazing. Animals are monitored weekly for FMD at the closest dip tank by the state veterinarian and animal health technicians.

The major water sources include the Pongolo River that drains the north-western area northwards toward the Usuthu River. The Usuthu River drains northwards into the Maputo River and ultimately the Indian Ocean just south of Maputo.

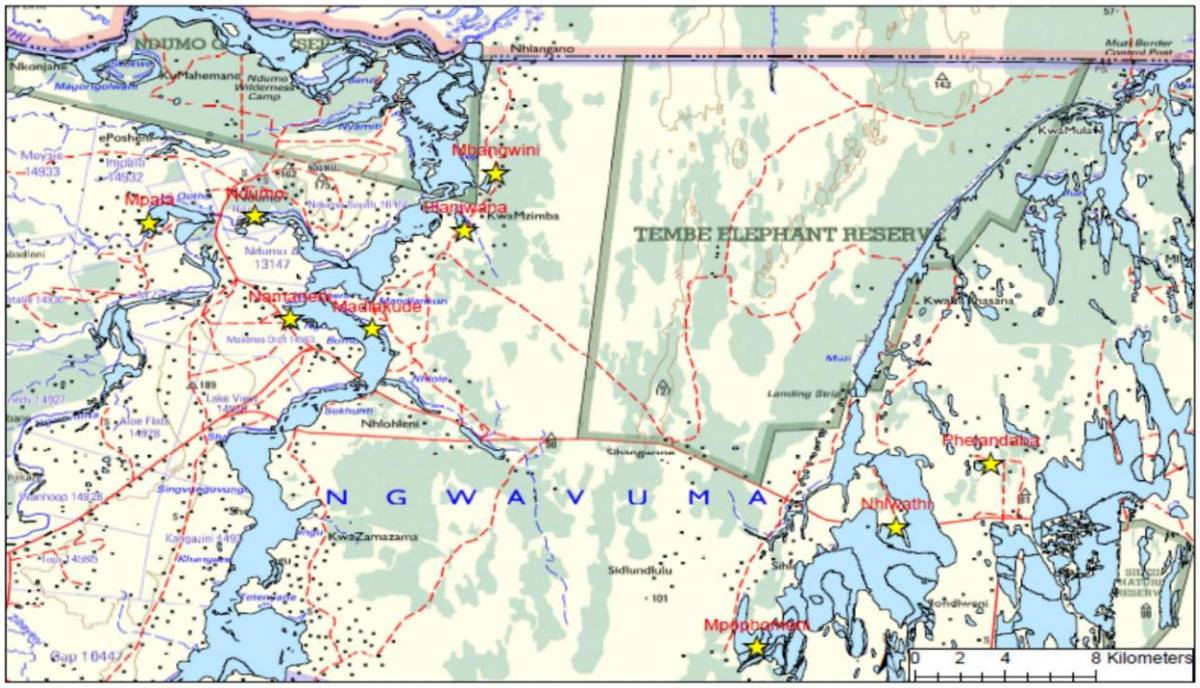


Figure 1.3: Ndumo area with dip tanks (yellow stars) for FMD control indicated. This map was constructed in Esri® ArcGIS 10.2 using country boundaries from Esri® ArcGIS Online, diptank coordinates collected during the study, and river, wetland (NFEPA, 2016) and protected area boundary (Daviews, 1991) data available under a Creative Commons Attribution (CC BY 4.0) license.

Figure 1.4: Map of KwaZulu-Natal with nature reserves, the block indicating the study area. https://www.roomsforafrica.com/dest/south-africa/kwazulu-natal.jsp

1.5 THESIS OUTLINE

Various reports have confirmed the occurrence of RVFV circulation during inter-epidemic periods in Africa on the basis of seropositivity in the absence of reported outbreaks or vaccination, in livestock (Rostal *et al*., 2010; Sumaye *et al*., 2013; Fafetine *et al*., 2013), wildlife (Britch *et al*., 2013; Fagbo *et al*., 2014) and humans (Olaleye *et al.,* 1996; LaBeaud *et al.,* 2008). A single reported longitudinal follow up study in Kenya, reported seroconversion in areas that had previously experienced outbreaks (Lichoti *et al*., 2014). However, no published studies have attempted to quantify the rate of seroconversion in livestock by follow-up of seronegative animals in a region where outbreaks have never been reported.

The objective of the first component of this study (Chapter 2) was to determine whether there was evidence of recent circulation of RVFV in the tropical region of far northern KZN Province; to estimate the seroprevalence in cattle and goats, and to determine the seroconversion rate in domestic ruminants over a two-year period. The results of this chapter were published in *PLoS Neglected Tropical Diseases*.

The objective of the second component (Chapter 3) was to determine the seroprevalence and associated risk factors of RVFV in antelope in the Tembe Elephant Park and the Ndumo Game Reserve, using sera from animals routinely culled over a two-year period. The results of this chapter have been submitted to *Transboundary and Emerging Infectious Diseases.*

The objective of the third component (Chapter 4) was to investigate the diversity, abundance, and seasonal dynamics of mosquitoes collected in the study area. Results from three localities were compared and mosquitoes were screened for the presence of RVFV.

Chapter 5 of the thesis reports the detection of RVFV, CHIKV and SINBV from a single pool of *Ae. durbanensis*.

REFERENCES

1. ABD, I. E.-R., ABD, U. E.-H. & HUSSEIN, M. 1999. An epizootic of Rift Valley fever in Egypt in 1997. *Revue scientifique et Technique (International Office of Epizootics),* 18**,** 741-748.
2. ABDEL AZIZ, M. 2008. Rift Valley Fever: The story unfolds…. *Sudanese Journal of Public Health,* 3**,** 5-10.
3. ABU-ELYAZEED, R., EL-SHARKAWY, S., OLSON, J., BOTROS, B., SOLIMAN, A., SALIB, A., CUMMINGS, C. & ARTHUR, R. 1996. Prevalence of anti-Rift-Valley-fever IgM antibody in abattoir workers in the Nile delta during the 1993 outbreak in Egypt. *Bulletin of the World Health Organization,* 74**,** 155.
4. ADAMS, M. J., LEFKOWITZ, E. J., KING, A. M., HARRACH, B., HARRISON, R. L., KNOWLES, N. J., KROPINSKI, A. M., KRUPOVIC, M., KUHN, J. H. & MUSHEGIAN, A. R. 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses. *Archives of Virology,* 162**,** 2505-2538.
5. ALEXANDER, R. 1951. Rift Valley fever in the Union. *Journal of the South African Veterinary Association,* 22**,** 105-112.
6. ANDRIAMANDIMBY, S. F., RANDRIANARIVO-SOLOFONIAINA, A. E., JEANMAIRE, E. M., RAVOLOLOMANANA, L., RAZAFIMANANTSOA, L. T., RAKOTOJOELINANDRASANA, T., RAZAINIRINA, J., HOFFMANN, J., RAVALOHERY, J.-P. & RAFISANDRATANTSOA, J.-T. 2010. Rift Valley fever during rainy seasons, Madagascar, 2008 and 2009. *Emerging Infectious Diseases,* 16**,** 963.
7. ARCHER, B. N., WEYER, J., PAWESKA, J., NKOSI, D., LEMAN, P., TINT, K. S. & BLUMBERG, L. 2011. Outbreak of Rift Valley fever affecting veterinarians and farmers in South Africa, 2008. *South African Medical Journal,* 101**,** 263-266.
8. ARISHI, H., AGEEL, A., RAHMAN, M. A., AL-HAZMI, A., ARISHI, A., AYOOLA, B., MENON, C., ASHRAF, J., FROGUSIN, O. & OCHIA, L. 2000. Update: outbreak of Rift Valley fever-Saudi Arabia, August-November 2000. *Morbidity and Mortality Weekly Report,* 49**,** 982-985.
9. BA, Y., SALL, A., DIALLO, D., MONDO, M., GIRAULT, L., DIA, I. & DIALLO, M. 2012. Re-emergence of Rift Valley fever virus in Barkedji (Senegal, West Africa) in 2002–2003: identification of new vectors and epidemiological implications. *Journal of the American Mosquito Control Association,* 28**,** 170-179.
10. BALKHY, H. H. & MEMISH, Z. A. 2003. Rift Valley fever: an uninvited zoonosis in the Arabian peninsula. *International Journal of Antimicrobial Agents,* 21**,** 153-157.
11. BARNARD, B. 1979. Rift Valley fever vaccine-antibody and immune response in cattle to a live and an inactivated vaccine. *Journal of the South African Veterinary Association,* 50**,** 155-157.
12. BENGIS, R., SWANEPOEL, R., DE KLERK, M., PIENAAR, N. & PRINSLOO, G 2010. Rift Valley fever: current concepts and recent findings. Proceedings of the 9th Annual Congress of the South African Society for Veterinary Epidemiology and Preventive Medicine. South African Society for Veterinary Epidemiology and Preventive Medicine, Pretoria, Republic of South Africa. Citeseer, 12-14.
13. BILLECOCQ, A., SPIEGEL, M., VIALAT, P., KOHL, A., WEBER, F., BOULOY, M. & HALLER, O. 2004. NSs protein of Rift Valley fever virus blocks interferon production by inhibiting host gene transcription. *Journal of Virology,* 78**,** 9798-9806.
14. BIRD, B. H., ALBARIÑO, C. G. & NICHOL, S. T. 2007a. Rift Valley fever virus lacking NSm proteins retains high virulence in vivo and may provide a model of human delayed onset neurologic disease. *Virology,* 362**,** 10-15.
15. BIRD, B. H., BAWIEC, D. A., KSIAZEK, T. G., SHOEMAKER, T. R. & NICHOL, S. T. 2007b. Highly sensitive and broadly reactive quantitative reverse transcription-PCR

assay for high-throughput detection of Rift Valley fever virus. *Journal of Clinical Microbiology,* 45**,** 3506-3513.
16. BIRD, B. H., KSIAZEK, T. G., NICHOL, S. T. & MACLACHLAN, N. J. 2009. Rift Valley fever virus. *Journal of the American Veterinary Medical Association,* 234**,** 883-893.
17. BIRD, B. H. & MCELROY, A. K. 2016. Rift Valley fever virus: Unanswered questions. *Antiviral research,* 132**,** 274-280.
18. CAPLEN, H., PETERS, C. & BISHOP, D. H. 1985. Mutagen-directed attenuation of Rift Valley fever virus as a method for vaccine development. *Journal of General Virology,* 66**,** 2271-2277.
19. CARROLL, S. A., REYNES, J.-M., KHRISTOVA, M. L., ANDRIAMANDIMBY, S. F., ROLLIN, P. E. & NICHOL, S. T. 2011. Genetic evidence for Rift Valley fever outbreaks in Madagascar resulting from virus introductions from the East African mainland rather than enzootic maintenance. *Journal of Virology,* 85**,** 6162-6167.
20. CÊTRE-SOSSAH, C., PÉDARRIEU, A., GUIS, H., DEFERNEZ, C., BOULOY, M., FAVRE, J., GIRARD, S., CARDINALE, E. & ALBINA, E. 2012. Prevalence of Rift Valley fever among ruminants, Mayotte. *Emerging Infectious Diseases,* 18**,** 972.
21. COACKLEY, W., PINI, A. & GOSDEN, D. 1967. Experimental infection of cattle with pantropic Rift Valley fever virus. *Research in Veterinary Science,* 8**,** 399-405.
22. COETZER, J. A. 1977. The pathology of Rift Valley fever. I. Lesions occurring in natural cases in new-born lambs. *Onderstepoort Journal of Veterinary Research,* 44, 205-212.
23. COETZER, J. A. 1982. The pathology of Rift Valley fever. II. Lesions occurring in field cases in adult cattle, calves and aborted foetuses. *Onderstepoort Journal of Veterinary Research,* 49, 11-17.
24. COETZER, J. A. & BARNARD, B. 1977. Hydrops amnii in sheep associated with hydranencephaly and arthrogryposis with Wesselsbron disease and Rift Valley fever viruses as aetiological agents. *Onderstepoort Journal of Veterinary Research*, 44, 119-126.
25. COLLETT, M. S., PURCHIO, A., KEEGAN, K., FRAZIER, S., HAYS, W., ANDERSON, D. K., PARKER, M. D., SCHMALJOHN, C., SCHMIDT, J. & DALRYMPLE, J. M. 1985. Complete nucleotide sequence of the M RNA segment of Rift Valley fever virus. *Virology,* 144**,** 228-245.
26. DAUBNEY, R. & HUDSON, J. 1933. Rift Valley fever. *East African Medical Journal,* 10**,** 2-19.
27. DAUBNEY, R., HUDSON, J. & GARNHAM, P. 1931. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep cattle and man from East Africa. *The Journal of Pathology and Bacteriology,* 34**,** 545-579.
28. DAVIES, F. & HIGHTON, R. 1980. Possible vectors of Rift Valley fever in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 74**,** 815-816.
29. DAVIES, F., LINTHICUM, K. & JAMES, A. 1985. Rainfall and epizootic Rift Valley fever. *Bulletin of the World Health Organization,* 63**,** 941.
30. DAVIES, A., DYE & DEANE. 1991. *Ezemvelo Protected Area boundary (ekznw_pabnd_2015_wdd.zip) [Internet]. Biodiversity Conservation Planning Division, Ezemvelo KZN Wildlife* [Online]. Available: http://www.sasdi.net/metaview.aspx?uuid=78e859b1c3161984b3e02739c58b3241. [Accessed].
31. DIGOUTTE, J., CORDELLIER, R., ROBIN, Y., PAJOT, F. & GEOFFROY, B. 1974. Le virus zinga (Ar B 1976), nouveau prototype d'arbovirus isolé en République Centrafricaine. *Annals of Microbiology.(Paris),* 125**,** 107-118.
32. DIGOUTTE, J. & PETERS, C. 1989. General aspects of the 1987 Rift Valley fever epidemic in Mauritania. *Research in Virology,* 140**,** 27-30.

33. DOHM, D. J., ROMOSER, W. S., TURELL, M. J. & LINTHICUM, K. J. 1991. Impact of stressful conditions on the survival of *Culex pipiens* exposed to Rift Valley fever virus. *Journal of the American Mosquito Control Association,* 7**,** 621-623.
34. DOHM, D. J., ROWTON, E. D., LAWYER, P. G., O'GUINN, M. & TURELL, M. J. 2000. Laboratory transmission of Rift Valley fever virus by *Phlebotomus duboscqi*, *Phlebotomus papatasi*, *Phlebotomus sergenti*, and *Sergentomyia schwetzi* (Diptera: Psychodidae). *Journal of Medical Entomology,* 37**,** 435-438.
35. DROSTEN, C., PANNING, M., GUENTHER, S. & SCHMITZ, H. 2002. False-negative results of PCR assay with plasma of patients with severe viral hemorrhagic fever. *Journal of Clinical Microbiology,* 40**,** 4394-4395.
36. EAGLESOME, M. & GARCIA, M. 1997. Disease risks to animal health from artificial insemination with bovine semen. *Revue Scientifique et Technique-Office International des épizooties,* 16**,** 215-225.
37. EASTERDAY, B., BENNETT, D. & MURPHY, L. 1962. Experimental Rift Valley fever in lambs and sheep. *American Journal of Veterinary Research,* 23**,** 1231.
38. EASTERDAY, B. C. 1965. Rift valley fever. *Advances in Veterinary Science,* 10**,** 65.
39. EL-AKKAD, A. 1978. Rift Valley fever outbreak in Egypt. October--December 1977. *The Journal of the Egyptian Public Health Association,* 53**,** 123.
40. ELHASSAN, T. M. A. 2006. *Epidemiology of Rift Valley Fever In Sudan.* University of Khartoum.
41. ELLIOTT, R. 2009. Bunyaviruses and climate change. *Clinical Microbiology and Infection,* 15**,** 510-517.
42. ELWAN, M., SHARAF, M., GAMEEL, K., EL-HADI, E. & ARTHUR, R. 1997. Rift valley fever retinopathy: Observations in a new outbreak. *Annals of Saudi Medicine,* 17**,** 377.
43. EVANS, A., GAKUYA, F., PAWESKA, J., ROSTAL, M., AKOOLO, L., VAN VUREN, P. J., MANYIBE, T., MACHARIA, J., KSIAZEK, T. & FEIKIN, D. 2008. Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife. *Epidemiology & Infection,* 136**,** 1261-1269.
44. FAFETINE, J.M., COETZEE, P., MUBEMBA, B., NHAMBIRRE, O., NEVES, L., COETZER, J.A. AND VENTER, E.H., 2016. Rift Valley fever outbreak in livestock, Mozambique, 2014. *Emerging Infectious Diseases*, 22(12), 2165 -2167.
45. FAFETINE, J., NEVES, L., THOMPSON, P. N., PAWESKA, J. T., RUTTEN, V. P. & COETZER, J. A. 2013. Serological evidence of Rift Valley fever virus circulation in sheep and goats in Zambezia Province, Mozambique. *PLoS Neglected Tropical Diseases,* 7**,** e2065.
46. FAFETINE, J. M., TIJHAAR, E., PAWESKA, J. T., NEVES, L. C., HENDRIKS, J., SWANEPOEL, R., COETZER, J. A., EGBERINK, H. F. & RUTTEN, V. P. 2007. Cloning and expression of Rift Valley fever virus nucleocapsid (N) protein and evaluation of a N-protein based indirect ELISA for the detection of specific IgG and IgM antibodies in domestic ruminants. *Veterinary Microbiology,* 121**,** 29-38.
47. FARAN, M. E., TURELL, M. J., ROMOSER, W. S., ROUTIER, R. G., GIBBS, P. H., CANNON, T. L. & BAILEY, C. L. 1987. Reduced survival of adult Culex pipiens infected with Rift Valley fever virus. *The American Journal of Tropical Medicine and Hygiene,* 37**,** 403-409.
48. FAYE, O., DIALLO, M., DIOP, D., BEZEID, O. E., BÂ, H., NIANG, M., DIA, I., MOHAMED, S. A. O., NDIAYE, K. & DIALLO, D. 2007. Rift Valley fever outbreak with East-Central African virus lineage in Mauritania, 2003. *Emerging Infectious Diseases,* 13**,** 1016.
49. FINDLAY, G. 1932. Rift Valley fever or enzootic hepatitis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 25**,** 229-IN11.
50. FINDLAY, G. & DAUBNEY, R. 1931. The virus of Rift Valley fever or enzootic hepatitis. *Lancet*.

51. FINDLAY, G. & HOWARD, E. 1951. Notes on Rift Valley fever. *Archives of Virology,* 4**,** 411-423.
52. FLICK, R. & BOULOY, M. 2005. Rift Valley fever virus. *Current Molecular Medicine,* 5**,** 827-834.
53. FOOD AND AGRICULTURE ORGANIZATION (FAO), 2003. Contingency plan.
54. FONTENILLE, D., TRAORE-LAMIZANA, M., DIALLO, M., THONNON, J., DIGOUTTE, J. & ZELLER, H. 1998. New vectors of Rift Valley fever in west Africa. *Emerging Infectious Diseases,* 4**,** 289.
55. FRANCIS JR, T. AND MAGILL, T.P., 1935. Rift Valley fever: a report of three cases of laboratory infection and the experimental transmission of the disease to ferrets. The Journal of experimental medicine, 62(3), 433.
56. GAD, A. M., FARID, H. A., RAMZY, R. R., RIAD, M. B., PRESLEY, S. M., COPE, S. E., HASSAN, M. M. & HASSAN, A. N. 1999. Host feeding of mosquitoes (Diptera: Culicidae) associated with the recurrence of Rift Valley fever in Egypt. *Journal of Medical Entomology,* 36**,** 709-714.
57. GARCIA, S., CRANCE, J. M., BILLECOCQ, A., PEINNEQUIN, A., JOUAN, A., BOULOY, M. & GARIN, D. 2001. Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. *Journal of Clinical Microbiology,* 39**,** 4456-4461.
58. GARGAN, T. P., JUPP, P. G. & NOVAK, R. 1988. Panveld oviposition sites of floodwater *Aedes* mosquitoes and attempts to detect transovarial transmission of Rift Valley fever virus in South Africa. *Medical and Ann. Microbiol Veterinary Entomology,* 2**,** 231-236.
59. GEAR, J., DE MEILLO, B., LE ROUX, A., KOFSKY, R., ROSE, R., STEYN, J., OLIFF, W. & SCHULZ, K. 1955. Rift Valley fever in South Africa: A study of the 1953 outbreak in the Orange Free State, with special reference: To the vectors and possible reservoir hosts. *South African Medical Journal,* 29.
60. GEAR, J., DE MEILLON, B., MEASROCH, V. & DAVIS, D. 1951. Rift Valley fever in South Africa: 2: the occurrence of human cases in the Orange Free State, the North-Western Cape Province, the Western And Southern Transvaal: B: field and laboratory investigations. *African Journal of Health Professions Education,* 25**,** 908-912.
61. GERDES, G. 2004. Rift Valley fever. *Revue scientifique et technique-Office International des Epizooties,* 23**,** 613-624.
62. GROBBELAAR, A. A., WEYER, J., LEMAN, P. A., KEMP, A., PAWESKA, J. T. & SWANEPOEL, R. 2011. Molecular epidemiology of Rift Valley fever virus. *Emerging Infectious Diseases,* 17**,** 2270.
63. GROLLA, A., MEHEDI, M., LINDSAY, R., BOSIO, C., DUSE, A. & FELDMANN, H. 2012. Enhanced detection of Rift Valley fever virus using molecular assays on whole blood samples. *Journal of Clinical Virology,* 54**,** 313-317.
64. HOCH, A. L., GARGAN II, T. P. & BAILEY, C. L. 1985. Mechanical transmission of Rift Valley fever virus by hematophagous Diptera. *The American Journal of Tropical Medicine and Hygiene,* 34**,** 188-193.
65. HOCH, A. L., TURELL, M. J. & BAILEY, C. L. 1984. Replication of Rift Valley fever virus in the sand fly Lutzomyia longipalpis. *The American Journal of Tropical Medicine and Hygiene,* 33**,** 295-299.
66. HOOGSTRAAL, H., MEEGAN, J. M., KHALIL, G. M. & ADHAM, F. K. 1979. The Rift Valley fever epizootic in Egypt 1977–1978 2. Ecological and entomological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73**,** 624-629.
67. IBRAHIM, M., TURELL, M., KNAUERT, F. & LOFTS, R. 1997. Detection of Rift Valley fever virus in mosquitoes by RT-PCR. *Molecular and Cellular Probes,* 11**,** 49-53.
68. IKEGAMI, T. 2012. Molecular biology and genetic diversity of Rift Valley fever virus. *Antiviral Research,* 95**,** 293-310.
69. IKEGAMI, T. & MAKINO, S. 2009. Rift valley fever vaccines. *Vaccine,* 27**,** D69-D72.

70. IKEGAMI, T. & MAKINO, S. 2011. The pathogenesis of Rift Valley fever. *Viruses,* 3**,** 493-519.
71. IKEGAMI, T., PETERS, C. & MAKINO, S. 2005. Rift valley fever virus nonstructural protein NSs promotes viral RNA replication and transcription in a minigenome system. *Journal of Virology,* 79**,** 5606-5615.
72. IKEGAMI, T., WON, S., PETERS, C. & MAKINO, S. 2007. Characterization of Rift Valley fever virus transcriptional terminations. *Journal of Virology,* 81**,** 8421-8438.
73. JEANMAIRE, E. M., RABENARIVAHINY, R., BIARMANN, M., RABIBISOA, L., RAVAOMANANA, F., RANDRIAMPARANY, T., FY ANDRIAMANDIMBY, S., DIAW, C. S., FENOZARA, P. & DE LA ROCQUE, S. 2011. Prevalence of Rift Valley fever infection in ruminants in Madagascar after the 2008 outbreak. *Vector-Borne and Zoonotic Diseases,* 11**,** 395-402.
74. JUPP, P. 2005. Mosquitoes as vectors of human disease in South Africa. *South African Family Practice,* 47**,** 68-72.
75. JUPP, P. & CORNEL, A. 1988. Vector competence tests with Rift Valley fever virus and five South African species of mosquito. *Journal of the American Mosquito Control Association,* 4**,** 4-8.
76. JUPP, P., GROBBELAAR, A., LEMAN, P., KEMP, A., DUNTON, R., BURKOT, T., KSIAZEK, T. & SWANEPOEL, R. 2000. Experimental detection of Rift Valley fever virus by reverse transcription-polymerase chain reaction assay in large samples of mosquitoes. *Journal of Medical Entomology,* 37**,** 467-471.
77. JUPP, P., KEMP, A., GROBBELAAR, A., LEMAN, P., BURT, F., ALAHMED, A., MUJALLI, D. A., KHAMEES, M. A. & SWANEPOEL, R. 2002. The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Medical and Veterinary Entomology,* 16**,** 245-252.
78. JUPP, P., MCINTOSH, B. & THOMPSON, D. 1984. Mechanical transmission of Rift Valley fever virus by mosquitoes. *South African Journal of Science*. No volume or page numbers
79. JUPP, P. G. 1996. *Mosquitoes of Southern Africa: culicinae and toxorhynchitinae*, Ekogilde Publishers.
80. KAKACH, L. T., WASMOEN, T. L. & COLLETT, M. S. 1988. Rift Valley fever virus M segment: use of recombinant vaccinia viruses to study Phlebovirus gene expression. *Journal of Virology,* 62**,** 826-833.
81. KASCHULA, v.R., 1953. The propagation and modification of strains of Rift Valley fever viruses in embryonated eggs and their use as immunizing agents for domestic ruminants. D.V.Sc. thesis, University of Pretoria.
82. KOKERNOT, R., HEYMANN, C., MUSPRATT, J. & WOLSTENHOLME, B. 1957. Studies on arthropod-borne viruses of Tongaland. V. Isolation of Bunyamwera and Rift Valley Fever viruses from mosquitoes. *South African Journal of Medical Sciences,* 22**,** 71-80.
83. KOKERNOT, R.H., SMITHBURN, K.C. AND KLUGE, E., 1961. Neutralizing antibodies against arthropod-borne viruses in the sera of domestic quadrupeds ranging in Tongaland, Union of South Africa*. Annals of Tropical Medicine & Parasitology*, 55(1), pp.73-85.
84. KORTEKAAS, J., ORESHKOVA, N., COBOS-JIMÉNEZ, V., VLOET, R. P., POTGIETER, C. A. & MOORMANN, R. J. 2011. Creation of a nonspreading Rift Valley fever virus. *Journal of Virology,* 85**,** 12622-12630.
85. LABEAUD, A. D., KAZURA, J. W. & KING, C. H. 2010. Advances in Rift Valley fever research: insights for disease prevention. *Current Opinion in Infectious Diseases,* 23**,** 403.
86. LE ROUX, C. A., KUBO, T., GROBBELAAR, A. A., VAN VUREN, P. J., WEYER, J., NEL, L. H., SWANEPOEL, R., MORITA, K. & PAWESKA, J. T. 2009. Development and evaluation of a real-time reverse transcription-loop-mediated isothermal

amplification assay for rapid detection of Rift Valley fever virus in clinical specimens. *Journal of Clinical Microbiology,* 47**,** 645-651.
87. LEDERBERG, J. 1993. Emerging infections: microbial threats to health. *Trends in Microbiology,* 1**,** 43-44.
88. LEFÈVRE, P.-C. La fièvre de la vallée du Rift. Annales de Médecine Vétérinaire, 1989. 453-463.
89. LINTHICUM, K., DAVIES, F., BAILEY, C. & KAIRO, A. 1983. Mosquito species succession in a dambo in an East African forest [Kenya]. *Mosquito News (USA)*.
90. LINTHICUM, K., DAVIES, F., BAILEY, C. & KAIRO, A. 1984a. Mosquito species encountered in a flooded grassland dambo in Kenya. *MOSQ. NEWS.,* 44**,** 228-232.
91. LINTHICUM, K., DAVIES, F. & KAIRO, A. 1984b. Observations of the biting activity of mosquitoes at a flooded dambo in Kenya. *Mosquito news*.
92. LINTHICUM, K., DAVIES, F., KAIRO, A. & BAILEY, C. 1985. Rift Valley fever virus (family *Bunyaviridae*, genus *Phlebovirus*). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Epidemiology & Infection,* 95**,** 197-209.
93. LINTHICUM, K. J., BRITCH, S. C. & ANYAMBA, A. 2016. Rift Valley fever: an emerging mosquito-borne disease. *Annual Eeview of Entomology,* 61**,** 395-415.
94. LINTHICUM, K. J., LOGAN, T. M., BAILEY, C. L., DOHM, D. J. & MOULTON, J. R. 1989. Transstadial and horizontal transmission of Rift Valley fever virus in Hyalomma truncatum. *The American Journal of Tropical Medicine and Hygiene,* 41**,** 491-496.
95. LOGAN, T., LINTHICUM, K., THANDE, P., WAGATEH, J. & ROBERTS, C. 1991. Mosquito species collected from a marsh in western Kenya during the long rains. *Journal of the American Mosquito Control Association,* 7**,** 395-399.
96. LOZACH, P.-Y., MANCINI, R., BITTO, D., MEIER, R., OESTEREICH, L., ÖVERBY, A. K., PETTERSSON, R. F. & HELENIUS, A. 2010. Entry of bunyaviruses into mammalian cells. *Cell Host & Microbe,* 7**,** 488-499.
97. LUTJEHARMS, J. & ROBERTS, H. 1988. The Natal pulse: An extreme transient on the Agulhas Current. *Journal of Geophysical Research: Oceans,* 93**,** 631-645.
98. LUTOMIAH, J., OMONDI, D., MASIGA, D., MUTAI, C., MIREJI, P. O., ONGUS, J., LINTHICUM, K. J. & SANG, R. 2014. Blood meal analysis and virus detection in blood-fed mosquitoes collected during the 2006–2007 Rift Valley fever outbreak in Kenya. *Vector-Borne and Zoonotic Diseases,* 14**,** 656-664.
99. MADANI, T. A., AL-MAZROU, Y. Y., AL-JEFFRI, M. H., MISHKHAS, A. A., AL-RABEAH, A. M., TURKISTANI, A. M., AL-SAYED, M. O., ABODAHISH, A. A., KHAN, A. S. & KSIAZEK, T. G. 2003. Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. *Clinical Infectious Diseases,* 37**,** 1084-1092.
100. MANSFIELD, K. L., BANYARD, A. C., MCELHINNEY, L., JOHNSON, N., HORTON, D. L., HERNÁNDEZ-TRIANA, L. M. & FOOKS, A. R. 2015. Rift Valley fever virus: A review of diagnosis and vaccination, and implications for emergence in Europe. *Vaccine,* 33**,** 5520-5531.
101. MCINTOSH, B. 1972. Rift Valley fever: 1. Vector studies in the field. *Journal of the South African Veterinary Association,* 43**,** 391-395.
102. MCINTOSH, B., JUPP, P., ANDERSON, D. & DICKINSON, D. 1973. Rift Valley fever. 2. Attempts to transmit virus with seven species of mosquito. *Journal of the South African Veterinary Association,* 44**,** 57-60.
103. MCINTOSH, B., JUPP, P., DOS SANTOS, I. & BARNARD, B. 1980a. Vector studies on Rift Valley fever virus in South Africa. *South African Medical Journal,* 58**,** 127-32.
104. MCINTOSH, B., RUSSELL, D. & GEAR, J. 1980b. Rift Valley fever in humans in South Africa. *South African Medical Journal* 58**,** 803-806.
105. MEEGAN, J. & BAILEY, C. 1989. Rift Valley Fever. In: MONATH, T.P., (ed.).*The Arboviruses: Epidemiology and Ecology*. Vol. lV. Boca Raton, Florida:CRC Press Inc. pp. 51-57.

106. MEEGAN, J., HOOGSTRAAL, H. & MOUSSA, M. 1979. An epizootic of Rift Valley fever in Egypt in 1977. *The Veterinary Record,* 105**,** 124-125.
107. MEEGAN, J. M. 1979. The Rift Valley fever epizootic in Egypt 1977–1978 1. Description of the epizootic and virological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73**,** 618-623.
108. MEEGAN, J. M. & SHOPE, R. E. 1981. Emerging concepts on Rift Valley fever. *Perspectives in Virology,* 11**,** 267-387.
109. MÉTRAS, R., CAVALERIE, L., DOMMERGUES, L., MÉROT, P., EDMUNDS, W. J., KEELING, M. J., CETRE-SOSSAH, C. & CARDINALE, E. 2016. The epidemiology of Rift Valley fever in Mayotte: insights and perspectives from 11 years of data. *PLoS Neglected Tropical Diseases,* 10**,** e0004783.
110. METRAS, R., PORPHYRE, T., PFEIFFER, D. U., KEMP, A., THOMPSON, P. N., COLLINS, L. M. & WHITE, R. G. 2012. Exploratory space-time analyses of Rift Valley fever in South Africa in 2008–2011. *PLoS Neglected Tropical Diseases,* 6**,** e1808.
111. MILLER, B. R., GODSEY, M. S., CRABTREE, M. B., SAVAGE, H. M., AL-MAZRAO, Y., AL-JEFFRI, M. H., ABDOON, A.-M. M., AL-SEGHAYER, S. M., AL-SHAHRANI, A. M. & KSIAZEK, T. G. 2002. Isolation and genetic characterization of Rift Valley fever virus from Aedes vexans arabiensis, Kingdom of Saudi Arabia. *Emerging Infectious Diseases,* 8**,** 1492.
112. MOIANE, B., MAPACO, L., THOMPSON, P., BERG, M., ALBIHN, A. AND FAFETINE, J., 2017. High seroprevalence of Rift Valley fever phlebovirus in domestic ruminants and African buffaloes in Mozambique shows need for intensified surveillance. *Infection Ecology & Epidemiology*, 7(1), p.1416248.
113. MORRILL, J., MEBUS, C. & PETERS, C. 1997a. Safety and efficacy of a mutagen-attenuated Rift Valley fever virus vaccine in cattle. *American Journal of Veterinary Research,* 58**,** 1104-1109.
114. MORRILL, J., MEBUS, C. & PETERS, C. 1997b. Safety of a mutagen-attenuated Rift Valley fever virus vaccine in fetal and neonatal bovids. *American Journal of Veterinary Research,* 58**,** 1110-1114.
115. MORRILL, J. & PETERS, C. 2003. Pathogenicity and neurovirulence of a mutagen-attenuated Rift Valley fever vaccine in rhesus monkeys. *Vaccine,* 21**,** 2994-3002.
116. MORVAN, J., SALUZZO, J.-F., FONTENILLE, D., ROLLIN, P. & COULANGES, P. 1991. Rift Valley fever on the east coast of Madagascar. *Research in Virology,* 142**,** 475-482.
117. MULLER, R., SALUZZO, J.-F., LOPEZ, N., DREIER, T., TURELL, M., SMITH, J. & BOULOY, M. 1995. Characterization of clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment. *The American Journal of Tropical Medicine and Hygiene,* 53**,** 405-411.
118. MUNDEL, B. & GEAR, J. 1951. Rift Valley Fever. I. The Occurrence of Human Cases in Johannesburg. *South African Medical Journal,* 25**,** 797-800.
119. MUNYUA P, MURITHI RM, WAINWRIGHT S, GITHINJI J, HIGHTOWER A, MUTONGA D, MACHARIA J, ITHONDEKA PM, MUSAA J, BREIMAN RF, BLOLAND P. Rift Valley fever outbreak in livestock in Kenya, 2006–2007. The American Journal of Tropical Medicine and Hygiene. 2010 Aug 5;83(2_Suppl):58-64.
120. NABETH, P., KANE, Y., ABDALAHI, M. O., DIALLO, M., NDIAYE, K., BA, K., SCHNEEGANS, F., SALL, A. & MATHIOT, C. 2001. Rift Valley fever outbreak, Mauritania, 1998: seroepidemiologic, virologic, entomologic, and zoologic investigations. *Emerging Infectious Diseases,* 7**,** 1052.
121. NFEPA. 2016. *Wetland Freshwater Ecosystem Priority Areas (NFEPA_Wetlands.shp) [Internet]. South African National Biodiversity Institute;* [Online]. Available: www.sasdi.net/metaview.aspx?uuid=330feba934dbd53fc832cee55768e188. [Accessed].

122. NJENGA, M. K., PAWESKA, J., WANJALA, R., RAO, C. Y., WEINER, M., OMBALLA, V., LUMAN, E. T., MUTONGA, D., SHARIF, S. & PANNING, M. 2009. Using a field quantitative real-time PCR test to rapidly identify highly viremic Rift Valley fever cases. *Journal of Clinical Microbiology,* 47**,** 1166-1171.
123. NYAKARAHUKA, L., BALINANDI, S., MULEI, S., KYONDO, J., TUMUSIIME, A., KLENA, J., LUTWAMA, J.J. AND SHOEMAKER, T., 2019. Ten outbreaks of rift valley fever in Uganda 2016-2018: Epidemiological and laboratory findings. *International Journal of Infectious Diseases*, 79, p.4.
124. ODENDAAL, L., FOSGATE, G. T., ROMITO, M., COETZER, J. A. & CLIFT, S. J. 2014. Sensitivity and specificity of real-time reverse transcription polymerase chain reaction, histopathology, and immunohistochemical labeling for the detection of Rift Valley fever virus in naturally infected cattle and sheep. *Journal of Veterinary Diagnostic Investigation,* 26**,** 49-60.
125. OIE 2012. *World Organisation for Animal Health Rift Valley fever. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. ,* Paris.
126. OLALEYE, O., TOMORI, O. & SCHMITZ, H. 1996. Rift Valley fever in Nigeria: infections in domestic animals. *Revue scientifique et technique-Office international des épizooties,* 15**,** 937-946.
127. OLIVE, M.-M., GOODMAN, S. M. & REYNES, J.-M. 2012. The role of wild mammals in the maintenance of Rift Valley fever virus. *Journal of Wildlife Diseases,* 48**,** 241-266.
128. PAWESKA, J. T., BURT, F. J., ANTHONY, F., SMITH, S. J., GROBBELAAR, A. A., CROFT, J. E., KSIAZEK, T. G. & SWANEPOEL, R. 2003. IgG-sandwich and IgM-capture enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in domestic ruminants. *Journal of Virological Methods,* 113**,** 103-112.
129. PAWESKA, J. T., BURT, F. J. & SWANEPOEL, R. 2005a. Validation of IgG-sandwich and IgM-capture ELISA for the detection of antibody to Rift Valley fever virus in humans. *Journal of Virological Methods,* 124**,** 173-181.
130. PAWESKA, J. T., MORTIMER, E., LEMAN, P. A. & SWANEPOEL, R. 2005b. An inhibition enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in humans, domestic and wild ruminants. *Journal of Virological Methods,* 127**,** 10-18.
131. PAWESKA, J. T., VAN VUREN, P. J., KEMP, A., BUSS, P., BENGIS, R. G., GAKUYA, F., BREIMAN, R. F., NJENGA, M. K. & SWANEPOEL, R. 2008. Recombinant nucleocapsid-based ELISA for detection of IgG antibody to Rift Valley fever virus in African buffalo. *Veterinary Microbiology,* 127**,** 21-28.
132. PAWESKA, J. T., VAN VUREN, P. J. & SWANEPOEL, R. 2007. Validation of an indirect ELISA based on a recombinant nucleocapsid protein of Rift Valley fever virus for the detection of IgG antibody in humans. *Journal of Virological Methods,* 146**,** 119-124.
133. PÉPIN, M. 2011. Rift Valley fever. *Medecine et Maladies Infectieuses,* 41**,** 322-329.
134. PEPIN, M., BOULOY, M., BIRD, B. H., KEMP, A. & PAWESKA, J. 2010. Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Veterinary Research,* 41**,** 61.
135. PEREZ, A. M., MEDANIC, R. C. & THURMOND, M. C. 2010. Rift Valley fever outbreaks in South Africa. *The Veterinary Record,* 166**,** 798.
136. PIENAAR, N. J. & THOMPSON, P. N. 2013. Temporal and spatial history of Rift Valley fever in South Africa: 1950 to 2011. *Onderstepoort Journal of Veterinary Research,* 80**,** 1-13.
137. RANDALL, R., GIBBS, C., AULISIO, C., BINN, L. & HARRISON, V. 1962. The development of a formalin-killed Rift Valley fever virus vaccine for use in man. *The Journal of Immunology,* 89**,** 660-671.
138. RATOVONJATO, J., OLIVE, M.-M., TANTELY, L. M., ANDRIANAIVOLAMBO, L., TATA, E., RAZAINIRINA, J., JEANMAIRE, E., REYNES, J.-M. & ELISSA, N. 2011.

Detection, isolation, and genetic characterization of Rift Valley fever virus from Anopheles (Anopheles) coustani, Anopheles (Anopheles) squamosus, and Culex (Culex) antennatus of the Haute Matsiatra region, Madagascar. *Vector-Borne and Zoonotic Diseases,* 11**,** 753-759.

139. ROGER, M., BERAL, M., LICCIARDI, S., SOULE, M., FAHAROUDINE, A., FORAY, C., OLIVE, M.-M., MAQUART, M., SOULAIMANE, A. & KASSIM, A. M. 2014. Evidence for circulation of the rift valley fever virus among livestock in the union of Comoros. *PLoS Neglected Tropical Diseases,* 8**,** e3045.
140. ROLIN, A. I., BERRANG-FORD, L. & KULKARNI, M. A. 2013. The risk of Rift Valley fever virus introduction and establishment in the United States and European Union. *Emerging Microbes & Infections,* 2**,** 1-8.
141. SALL, A., MACONDO, E., SENE, O., DIAGNE, M., SYLLA, R., MONDO, M., GIRAULT, L., MARRAMA, L., SPIEGEL, A. & DIALLO, M. 2002. Use of reverse transcriptase PCR in early diagnosis of Rift Valley fever. *Clinical Diagnostics and Laboratory Immunology,* 9**,** 713-715.
142. SANG, R., KIOKO, E., LUTOMIAH, J., WARIGIA, M., OCHIENG, C., O'GUINN, M., LEE, J. S., KOKA, H., GODSEY, M. & HOEL, D. 2010. Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations. *The American Journal of Tropical Medicine and Hygiene,* 83**,** 28-37.
143. SCHMALJOHN, C. & NICHOL, S. 2007. Bunyaviridae, p 1741–1790. *Fields Virology. Lippencott Williams & Wilkins, Philadelphia, PA*.
144. SCOTT, G., COACKLEY, W., ROACH, R. & COWDY, N. 1963. Rift Valley fever in camels. *The Journal of Pathology and Bacteriology,* 86**,** 229-231.
145. SEUFI, A. M. & GALAL, F. H. 2010. Role of Culex and Anopheles mosquito species as potential vectors of rift valley fever virus in Sudan outbreak, 2007. *BMC Infectious Diseases,* 10**,** 65.
146. SHIMSHONY, A. & BARZILAI, R. 1983. Rift valley fever. *Advances in Veterinary Science and Comparative Medicine,* 27**,** 347.
147. SHOEMAKER, T., BOULIANNE, C., VINCENT, M. J., PEZZANITE, L., AL-QAHTANI, M. M., AL-MAZROU, Y., KHAN, A. S., ROLLIN, P. E., SWANEPOEL, R. & KSIAZEK, T. G. 2002. Genetic analysis of viruses associated with emergence of Rift Valley fever in Saudi Arabia and Yemen, 2000-01. *Emerging Infectious Diseases,* 8**,** 1415.
148. SIAM, A., MEEGAN, J. & GHARBAWI, K. 1980. Rift Valley fever ocular manifestations: observations during the 1977 epidemic in Egypt. *British Journal of Ophthalmology,* 64**,** 366-374.
149. SMITHBURN, K. 1949. Rift Valley fever: the neurotropic adaptation of the virus and the experimental use of this modified virus as a vaccine. *British Journal of Experimental Pathology,* 30**,** 1.
150. SMITHBURN, K., HADDOW, A. & GILLETT, J. 1948. Rift Valley fever. Isolation of the virus from wild mosquitoes. *British Journal of Experimental Pathology,* 29**,** 107.
151. SPIK, K., SHURTLEFF, A., MCELROY, A. K., GUTTIERI, M. C., HOOPER, J. W. & SCHMALJOHN, C. 2006. Immunogenicity of combination DNA vaccines for Rift Valley fever virus, tick-borne encephalitis virus, Hantaan virus, and Crimean Congo hemorrhagic fever virus. *Vaccine,* 24**,** 4657-4666.
152. STEYN, J. & SCHULZ, K. 1955. Aëdes (Ochlerotatus) caballus Theobald, the South African vector of Rift Valley fever. *South African Medical Journal,* 29**,** 1114-20.
153. SUZICH, J. A. & COLLETT, M. S. 1988. Rift Valley fever virus M segment: cell-free transcription and translation of virus-complementary RNA. *Virology,* 164**,** 478-486.
154. SWANEPOEL, R. Observations on Rift Valley fever in Zimbabwe. *Contributions to Epidemiology and Biostatistics*, 3: Rift Valley fever, 83-91.
155. SWANEPOEL, R. & COETZER, J. 2004. Rift valley fever. *Infectious Diseases of Livestock,* 2**,** 1037-1070.

156. SWANEPOEL, R., STRUTHERS, J., ERASMUS, M., SHEPHERD, S., MCGILLIVRAY, G., ERASMUS, B. & BARNARD, B. 1986. Comparison of techniques for demonstrating antibodies to Rift Valley fever virus. *Epidemiology & Infection,* 97**,** 317-329.
157. TORRES-VÉLEZ, F. & BROWN, C. 2004. Emerging infections in animals--potential new zoonoses? *Clinics in Laboratory Medicine,* 24**,** 825-38, viii.
158. TURELL, M. J., LINTHICUM, K. J., PATRICAN, L. A., DAVIES, F. G., KAIRO, A. & BAILEY, C. L. 2008. Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley fever virus. *Journal of Medical Entomology,* 45**,** 102-108.
159. TURELL, M. J. & PERKINS, P. V. 1990. Transmission of Rift Valley fever virus by the sand fly, Phlebotomus duboscqi (Diptera: Psychodidae). *The American Journal of Tropical Medicine and Hygiene,* 42**,** 185-188.
160. VAN VUREN, P. J., POTGIETER, A. C., PAWESKA, J. T. & VAN DIJK, A. A. 2007. Preparation and evaluation of a recombinant Rift Valley fever virus N protein for the detection of IgG and IgM antibodies in humans and animals by indirect ELISA. *Journal of Virological Methods,* 140**,** 106-114.
161. VIALAT, P., BILLECOCQ, A., KOHL, A. & BOULOY, M. 2000. The S segment of rift valley fever phlebovirus (Bunyaviridae) carries determinants for attenuation and virulence in mice. *Journal of Virology,* 74**,** 1538-1543.
162. WATTS, D., PANTUWATANA, S., DEFOLIART, G., YUILL, T. & THOMPSON, W. 1973. Transovarial transmission of LaCrosse virus (California encephalitis group) in the mosquito, Aedes triseriatus. *Science,* 182**,** 1140-1141.
163. WEINBREN, M., WILLIAMS, M. & HADDOW, A. 1956. Isolation of an atypically reacting strain of Rift Valley fever virus from Lunyo, on the Entebbe Peninsula. East African Virus Research Institute Report, July 1955–June 1956. *Publication of the East African High Commission. Nairobi, Kenya: Government Printer***,** 21-26.
164. WILLIAMS, M., WOODALL, J., CORBET, P. & HADDOW, A. 1960. An outbreak of Rift Valley fever occurring near Entebbe: Entomological studies and further isolations. *East African Virus Research Institute Reports,* 10**,** 24-25.
165. WILSON, M. L., CHAPMAN, L. E., HALL, D. B., DYKSTRA, E. A., BA, K., ZELLER, H. G., TRAORE-LAMIZANA, M., HERVY, J.-P., LINTHICUM, K. J. & PETERS, C. 1994. Rift Valley fever in rural northern Senegal: human risk factors and potential vectors. *The American Journal of Tropical Medicine and Hygiene,* 50**,** 663-675.
166. WON, S., IKEGAMI, T., PETERS, C. & MAKINO, S. 2007. NSm protein of Rift Valley fever virus suppresses virus-induced apoptosis. *Journal of Virology,* 81**,** 13335-13345.
167. ZAKI, A., COUDRIER, D., YOUSEF, A., FAKEEH, M., BOULOY, M. & BILLECOCQ, A. 2006. Production of monoclonal antibodies against Rift Valley fever virus: Application for rapid diagnosis tests (virus detection and ELISA) in human sera. *Journal of Virological Methods,* 131**,** 34-40.

168. OIE 2009. Rift Valley fever, in manual of diagnostic tests and vaccines for terrestrial animals (http://www.oie.int/eng/en_index.html).

Chapter 2

High seroconversion rate to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal, South Africa, in the absence of reported outbreaks

This chapter was published: van den Bergh, C., Venter, E.H., Swanepoel, R. and Thompson, P.N., 2019. High seroconversion rate to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal, South Africa, in the absence of reported outbreaks. *PLoS Neglected Tropical Diseases*, *13*(5), e0007296.

Abstract

Rift Valley fever is a mosquito-borne zoonotic disease characterized in South Africa by large epidemics amongst ruminant livestock at very long, irregular intervals, mainly in the central interior. However, the presence and patterns of occurrence of the virus in the eastern parts of the country are poorly known. This study aimed to detect the presence of RVFV in cattle and goats in far northern KZN Province and to estimate the prevalence of antibodies to the virus and the incidence rate of seroconversion.

Cross-sectional studies were performed in communally farmed cattle (n=423) and goats (n=104), followed by longitudinal follow-up of seronegative livestock (n=253) 14 times over 24 months, representing 160.3 animal-years at risk. Exposure to RVFV was assessed using an IgG sandwich ELISA and a SNT and seroconversion was assessed using SNT. Incidence density was estimated and compared using multivariable Poisson models and hazard of seroconversion was estimated over time.

Initial overall seroprevalence was 34.0% (95%CI: 29.5-38.8%) in cattle and 31.7% (95%CI: 22.9-41.6%) in goats, varying by locality from 18-54%. Seroconversions to RVFV based on SNT were detected throughout the year, with the incidence rate peaking during the high rainfall months of January to March and differed considerably between years. Overall seroconversion rate in cattle was 0.59 per animal-year (95% CI: 0.46-0.75) and in goats it was 0.41 per animal-year (95% CI: 0.25-0.64), varying significantly over short distances.

The high seroprevalence in all age groups and evidence of year-round viral circulation provide evidence for a hyperendemic situation in the study area. This is the first study to directly estimate infection rate of RVFV in livestock in an endemic area in the absence of reported outbreaks and provides the basis for further investigation of factors affecting viral circulation and mechanisms for virus survival during interepidemic periods.

Author summary:

Rift Valley fever is a mosquito-transmitted viral disease that may cause large epidemics in domestic livestock and in humans. Although currently largely confined to Africa, it is of international concern due to its ability to spread and become established in areas where suitable mosquito carriers occur. Outbreaks occur sporadically, associated with conditions favourable for proliferation of mosquito populations, such as high rainfall and flooding, yet their location and timing remain difficult to predict. In other areas there is evidence that RVF virus is endemic and may circulate without causing outbreaks. However, the location and extent of such areas is poorly known, as is the transmission dynamics of the virus in those areas. In this paper, we report the existence of such an area of endemic RVF virus transmission on the tropical coastal plain of South Africa bordering Mozambique, where we found a high rate of exposure of domestic cattle and goats to the naturally circulating virus over a two-year period, with no outbreaks being reported. Research in such areas will help us to assess the potential for spread of the virus to other areas and also to better understand the behaviour of the virus during periods between epidemics.

2.1 INTRODUCTION

Rift Valley fever is a zoonotic arboviral disease caused by RVFV), a *Phlebovirus* in the family *Phenuiviridae* (Adams *et al*., 2017), mainly affecting livestock and humans and transmitted by *Aedes* and *Culex* spp. mosquitoes. Non-vector transmission of RVFV is not considered important in livestock but humans are easily infected by contact with bodily fluids of infected animals or by inhaling infectious particles (Swanepoel and

Coetzer, 2004). Human infection may present in a self-limiting febrile disease with signs including fever, severe headache, malaise, muscle pain and nausea, but in severe cases can result in encephalopathy, haemorrhagic signs, retinopathy and even death. The disease in ruminants may be characterized by necrotic hepatitis but may also be inapparent or mild, and usually results in abortions and neonatal mortalities in pregnant animals (Swanepoel and Coetzer, 2004).

The virus was first isolated in Kenya in 1930 (Daubney *et al*., 1931), and has been endemic in sub-Saharan Africa ever since. The virus spread beyond sub-Saharan Africa when outbreaks occurred in the Nile delta in Egypt during 1977-1978 causing mortalities in both humans and livestock (Laughlin *et al*., 1979, Meegan, 1979). It was first recorded in Mauritania and Senegal in West Africa in 1987 (Ksiazek *et al*., 1989) and spread beyond mainland Africa, to Saudi Arabia and Yemen in 2000 (Jupp *et al*., 2000, Shoemaker *et al*., 2002, Balkhy and Memish, 2003), the Comoros in 2007 (Sissoko *et al*., 2009) and Madagascar in 1990 (Morvan *et al*., 1991). Three major RVF epidemics have occurred in South Africa, during 1950-1951, 1974-1975 and more recently 2008-2011 (Pienaar and Thompson, 2013). During the 2010 outbreaks a total of 302 human infections were diagnosed with a case fatality rate of 8% (Archer *et al*., 2013) and a loss of 19,000 head of livestock was reported. Species such as buffaloes, camels and other wildlife were also affected (Glancey *et al*., 2015). These large epidemics have occurred mainly following unusually heavy rains on the relatively dry central plateau of South Africa. However, in the periods between these large epidemics, several smaller outbreaks or isolated cases have occurred both in the interior and in the eastern parts of the country (Pienaar and Thompson, 2013).

The fate of the virus during the long IEP in South Africa is poorly understood. The virus has been isolated from newly emerged, unfed *Aedes lineatopennis* (*Ae. mcintoshi*) in East Africa, suggesting that it may survive extended periods in aedine mosquito eggs, which are very resilient in dry conditions (Linthicum *et al*., 1985). Romoser *et al.* (*et al*(2011) provided laboratory evidence in support of transovarial transmission in artificially infected *Ae. mcintoshi*, and it remains the most popular theory for interepidemic survival of RVFV; however, evidence for its occurrence in nature is limited. Other possibilities are the occurrence of low-level inter-epidemic circulation of

virus between vectors and unknown hosts in endemic areas, or disappearance of virus from an area with later re-introduction from an endemic area via movement of infected hosts or vectors (Swanepoel and Coetzer, 2004).

One such endemic area may be the low-lying, tropical eastern part of southern Africa, including Mozambique and small parts of north-eastern South Africa. Very few outbreaks have been reported in Mozambique, yet there is serological evidence of widespread exposure to RVFV in livestock (Fafetine *et al*., 2013) and humans (Niklasson *et al*., 1987). RVFV was responsible for a small outbreak in goats in the Maputo Province, close to the South African border, in 2014 (Fafetine *et al*., 2016). Recently, the overall seroprevalence of RVFV antibodies in seven provinces of Mozambique was reported to be 37% in cattle, 30% in African buffalo (*Syncerus caffer*), and 29% in domestic ruminants in the Maputo Province (Moiane *et al*., 2017). In South Africa, RVFV was isolated from *Ae. circumluteolus* in the tropical coastal region of far northern KZN in 1955 (Kokernot *et al*., 1957a) and at the same time neutralizing antibodies to RVFV were found in 12% of domestic livestock (Kokernot *et al*., 1962) and 10% of humans (Smithburn *et al*., 1959). However, subsequent efforts to detect the virus in mosquitoes in that region were unsuccessful (McIntosh, 1972). There have also not been any reports of clinical cases of RVF in that region, although further south along the KZN coast there is some evidence of RVFV circulation and sporadic clinical cases in 1972-1973 (McIntosh *et al*., 1980) and in a dairy herd in 1981 (McIntosh *et al*., 1983).

Various reports have inferred the occurrence of interepidemic circulation of RVFV on the basis of seropositivity in the absence of reported outbreaks or vaccination, in livestock (Fafetine *et al*., 2013, Rostal *et al*., 2010, Sumaye *et al*., 2013) wildlife (Britch *et al*., 2013, Fagbo *et al*., 2014) and humans (Olaleye *et al*., 1996, LaBeaud, 2008). Two recent studies have reported longitudinal follow-up of sentinel sheep and goats in Kenya, reporting low rates of seroconversion in areas that had previously experienced outbreaks (Lichoti *et al*., 2014, Mbotha *et al*., 2018). However, no published studies have attempted to quantify the rate of seroconversion in livestock by follow-up of seronegative animals in a region where outbreaks have never been reported.

The objectives of this study were to determine whether there was evidence of recent circulation of RVFV in the tropical region of far northern KZN, South Africa, to estimate the seroprevalence in cattle and goats, and to estimate the incidence rate and patterns of seroconversion in domestic ruminants over a two-year period.

2.2 MATERIALS AND METHODS

The study protocol was approved by the Animal Ethics Committee of the University of Pretoria (V013-16) and the Department of Agriculture, Forestry and Fisheries, Republic of South Africa, and adhered to the specifications of the South African National Standard (SANS 10386-2008): “The Care and Use of Animals for Scientific Purposes”. Support for the project was obtained from the Director of Veterinary Services of KZN and the State Veterinarian: Jozini. Verbal consent was obtained from animal owners after the aims of the study were explained to them by the State-employed animal health technicians. Farmers were rewarded for their participation by providing them with small amounts of animal health products such as wound aerosol, topical treatments and endoparasiticides.

2.2.1 Study area

The study was conducted on the Maputaland coastal plain in far northern KZN just south of the Mozambique border, an area with a tropical climate (Bruton, 1980) characterised by warm, dry winters and hot, wet summers (Fig. 2.1). In the Pongolo River floodplain, the core of the study area, the summer temperatures range from 23-40°C, winter temperatures range from 16-26°C and mean annual rainfall is 600-800 mm (Jozini Municipality, 2017). The northwards flowing Pongolo River forms a floodplain at an elevation of 30-50 m above sea level, approximately 10-15 km to the east of the foothills of the Lebombo Mountains, which rise to about 650 m above sea level. The study area contains a mosaic of bushland, thicket, wooded grassland, riverine forest and floodplain vegetation (Moll, 1980). Depending on rainfall, seasonal flooding of the Pongolo, Ingwavuma and Usuthu Rivers inundates about 13,000 ha of

floodplain, filling numerous pans, some of which retain water during the dry season (Rogers, 1980). The construction of the Pongolapoort Dam in 1973 largely disrupted this cycle, with flooding only occurring with heavy rainfalls or with periodic opening of sluice gates. In very dry years, permanent water may only be present in the Pongolo and Usuthu Rivers and in very few of the larger pans. This study started during a drought period when most of the pans were dry (Fig. 2.2).

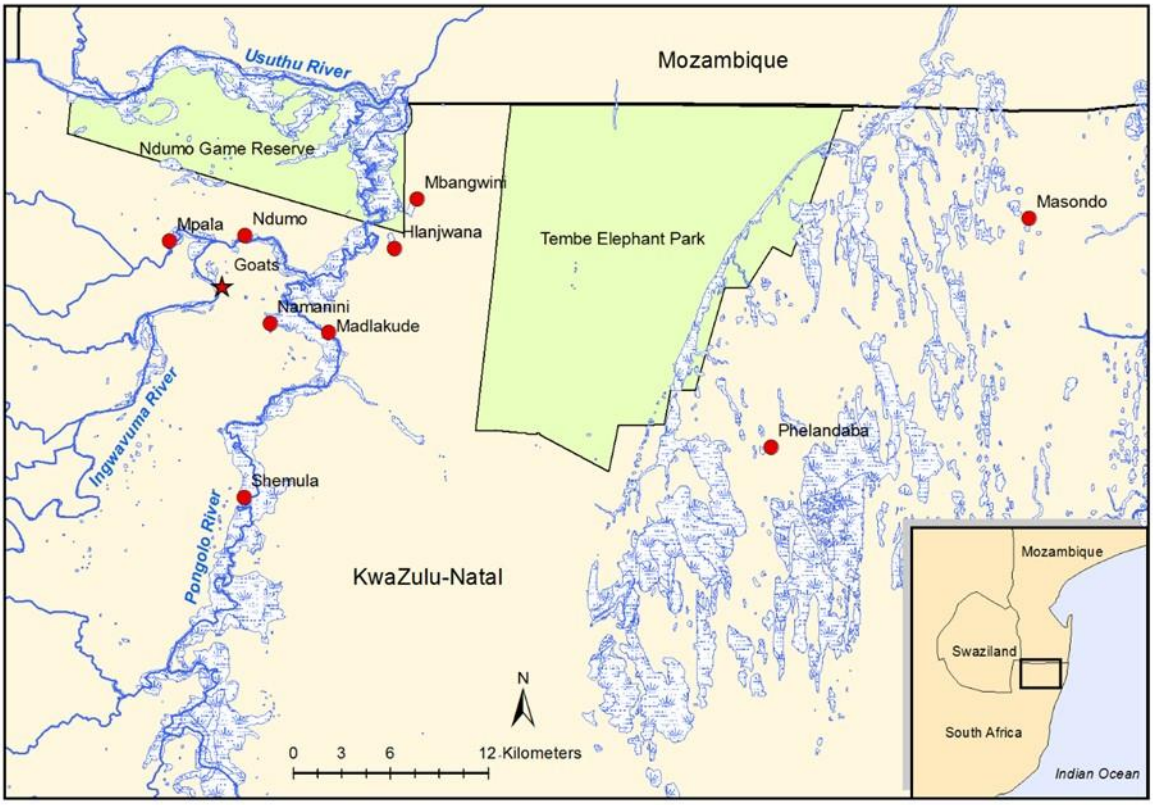


Figure 2.1. A map of the study area showing major rivers and temporary wetlands, with the locations of the nine diptanks where cattle were sampled and the area where goats were sampled for the cross-sectional study. The map was constructed for this manuscript in Esri® ArcGIS 10.2 using country boundaries from Esri® ArcGIS Online, diptank coordinates collected during the study, and river (River_FEPAs.shp), wetland (NFEPA_Wetlands.shp) and protected area boundary (ekznw_pabnd_2015_wdd.zip) data available under a Creative Commons Attribution (CC BY 4.0) license.

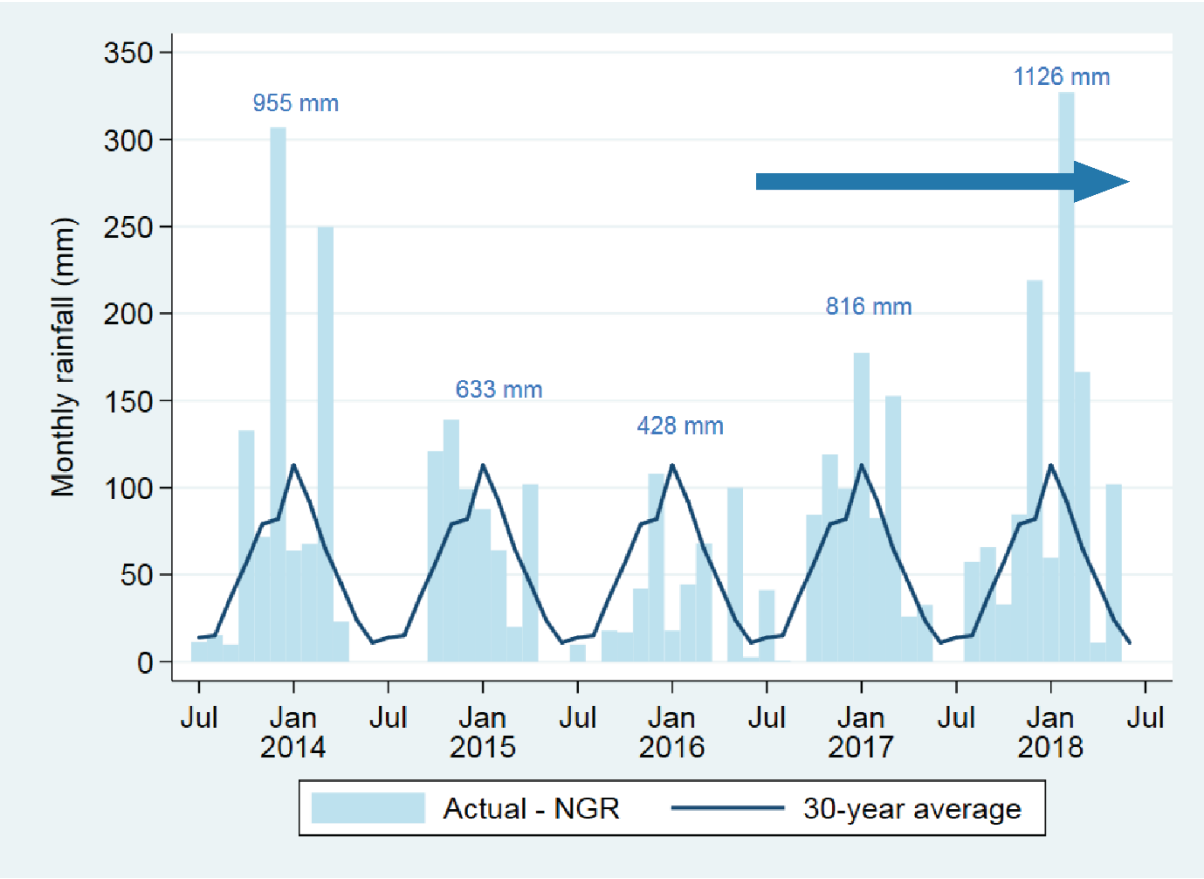


Figure 2.2. Actual monthly rainfall recorded at Ndumo Game Reserve (NGR) in the study area, with 30-year average. Totals above the bars indicate rainfall for each year (July - June). The blue arrow indicates the study period (June 2016 - June 2018).

Livestock in the study area (mainly cattle and goats) belong to numerous small-scale farmers and are communally grazed. Animals range widely during the day, during the dry season foraging mainly in the floodplains, and may be confined in pens at night. Much of the area lies within a FMD protection zone and cattle are brought weekly (summer) or every two weeks (winter) to dip tanks where they are inspected by animal health technicians for signs of FMD and plunge-dipped for ectoparasite control using amitraz. For this inspection purposes, most cattle are individually identified using ear tags. The only vaccinations applied are those provided by the State, namely brucellosis, anthrax, blackquarter and sometimes lumpy skin disease vaccines.

2.2.2 Study design and sampling

The study took the form of an initial cross-sectional survey of individually identified cattle at nine dip tanks (Fig. 2.1) during June 2016, followed by a longitudinal follow-up of seronegative cattle to detect and quantify seroconversion. The nine dip tanks were selected on the basis of their location adjacent to the floodplain of the Pongolo River and its tributaries (7 dip tanks) and the eastern coastal plain (2 dip tanks), as well as the willingness of farmers and animal health technicians to participate.

Of the 7 dip tanks in the western part of the study area, all were situated within 1.5 km of the Pongolo River, except for Mpala which was adjacent to a small tributary which held water for almost the entire study period and Ndumo which was about 4 km from the Pongolo River and adjacent to the Ingwavuma River which remained dry throughout the study period. The 2 dip tanks in the eastern part of the study area were situated in a very sandy area with a mosaic of grassland and bush and with numerous small temporary pans during the wet season. The minimum distance between diptanks, were ±5 km which was the distance between Mpala and Ndumo diptank.

Sample size (n) was calculated in order to detect a seroprevalence (P_{exp}) of 25% with an allowable error (d) of 10% and 95% confidence, using the formula $n = 1.96^2 P_{exp}(1 - P_{exp}) / d^2$ (Thrusfield, 2018). The required sample size of 73 was then multiplied by a design effect (D) of 5.9, calculated by assuming an intracluster correlation coefficient (ρ) of 0.1 and a cluster size (m) of 50 animals per dip tank, using the formula $D = 1 + \rho(m - 1)$ (Bennett *et al*., 1991); the overall required sample size was therefore 432.

Animals were selected using systematic random sampling, selecting 2-5 animals per farmer at each dip tank as they passed through a crush either before or after dipping. Only cattle >6 months old were sampled. Following serological testing of samples from the cross-sectional study, as many seronegative animals as possible, as well as a small number of seropositive animals, were then re-sampled during 13 sampling episodes at 1-2-month intervals between November 2016 and June 2018. To compensate for loss to follow-up, some additional cattle from the same owners were recruited at various times during the study.

A smaller study of the same type was conducted in goats in an area close to one of the dip tanks (Fig. 2.1); 104 goats belonging to 7 farmers were sampled during February to April 2017 and seronegative animals were re-sampled at 1-2 monthly intervals until June 2018.

Blood samples were collected from the jugular vein of goats and coccygeal vein of cattle. Samples were refrigerated and transported to a BSL2+ laboratory at the Faculty of Veterinary Science, University of Pretoria, where they were centrifuged. The sera were inactivated at 56°C for 1 hour and stored at -20°C until used.

2.2.3 Serology

All cattle serum samples that were collected for the cross-sectional study were tested for RVFV IgG antibodies using an IgG sandwich ELISA with reported sensitivity of 96.3% and specificity of 99.7% in cattle (Paweska *et al*., 2003). In addition, approximately every second sample (due to economic constraints) was tested using the SNT. Where possible, samples that tested positive for RVFV IgG were also tested for IgM antibodies using an IgM capture ELISA with reported sensitivity of 100% in sheep and specificity of 99.2% in cattle (Paweska *et al*., 2003). In order to detect seroconversion, all samples collected during the longitudinal study, as well as all goat serum samples, were tested using SNT.

Heat-inactivated serum samples were tested by the IgG sandwich ELISA using a previously described and validated method (Paweska *et al*., 2003). F96 Maxisorp Immunoplates (AEC-Amersham) were coated with anti-RVF hyperimmune mouse ascitic fluid. Optical densities (OD) were measured at 450 nm. The net OD values were first recorded for each serum as the value determined with the RVFV antigen minus the value determined with the control antigen and subsequently converted into percentage of the OD value of a high positive control serum. A cut-off value optimized for this assay (PP value ≥30) was used.

The IgM capture ELISA was performed following a method previously described (Paweska *et al*., 2003). Briefly: plates were coated overnight at 4°C with 100 µl rabbit

anti-sheep IgM. OD was determined at 450 nm. The PP was determined as above and an optimized cut-off value of PP≥30 was used.

The SNT was performed in 96-well plate (AEC-Amersham) format according to the standard protocol of the Virology Section, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, which follows the method prescribed by the World Organisation for Animal Health (OIE, 2012). Briefly: Sera were diluted 1:5 in PBS+ (phosphate buffered saline with added $MgCl_2$ and $CaCl_2$) and two-fold dilutions of the serum were made. The $TCID_{50}$ (Smithburn vaccine strain) was determined using the Karber method (Karber, 1931). A volume of 100 $TCID_{50}$ virus was added to each dilution and incubated at 37°C for 60 min. A total of 80 µl of African green monkey kidney cells (Vero) (480,000 cells/mL) in MEM containing 5% foetal calf serum (Biowest, Celtic) was added to each well. The microplates were incubated at 37°C in an atmosphere containing 5% CO_2 and observed daily for cytopathic effect; the titre was taken as the dilution at which 50% of the cells were affected. Results were only accepted if all controls gave the expected results (virus control, positive serum and negative serum). A serum dilution of ≥1:10 was used to define seropositivity.

2.2.4 Statistical analysis

For samples tested during the cross-sectional study using both the IgG ELISA and the SNT, sensitivity and specificity of the IgG ELISA were estimated, with exact binomial 95% confidence intervals, using the SNT as the gold standard. For animals on which both tests were used, the SNT result was used to define seropositivity, otherwise the IgG ELISA result was used. The RVFV seroprevalence in cattle in June 2016 was calculated overall and by dip tank, age group (≤2 y, 2.5-3.5 y, 4-5.5 y, ≥6 y) and sex. For goats the seroprevalence in February 2017 was calculated overall and by age group and sex. Exact binomial 95% confidence intervals were calculated and seroprevalence was compared between dip tanks (cattle only), age groups and sexes using multiple logistic regression to control for confounding.

The occurrence of seroconversion was defined as a seronegative result (either during the cross-sectional study as defined above, or during subsequent testing by SNT) followed by a sample from the same animal that tested positive by SNT at a serum dilution of 1:10 or greater. The incidence rate of seroconversion over the 24-month study period (16 months in goats) was then calculated by including all animals that were sampled more than once (irrespective of date of first sampling) and that were seronegative on the first sampling. For the animals that did not seroconvert, animal-time at risk was calculated as the interval between the first and last tests. For animals that did seroconvert, the day of seroconversion was unknown, but was assumed to be the midpoint between the last negative and first positive test. The seroconversion rate was calculated as the number of seroconversions divided by the total animal-years at risk observed, with Poisson exact 95% confidence limits. Estimates were calculated overall for each species, separately for each year of the study (June 2016 to June 2017 and July 2017 to June 2018) and for cattle also separately for each dip tank. Associations of age, sex and place with incidence rate were then assessed by including these variables in a multiple Poisson regression model, using animal-time at risk as the exposure variable. Assessment of overdispersion in the outcome was done by fitting the corresponding negative binomial model and determining the significance of the overdispersion parameter, α.

For each species, the seroconversion rate with 95% confidence interval was plotted over time as the derivative of the kernel-smoothed Nelson-Aalen cumulative hazard estimator, using a 60-day kernel width. For this purpose, instead of the midpoint-imputed seroconversion date used above, a randomly selected date between the last negative and first positive test was used for seroconverting animals in order to avoid systematic bias in incidence rate estimation over time (Vandormael *et al*., 2018). All statistical analyses were done using Stata 15 (StataCorp, College Station, TX, U.S.A) and statistical significance was assessed at $P<0.05$. Risk surfaces for seroprevalence and seroconversion rate in the study area were created by interpolation using ordinary kriging in ArcGIS 10.2 (Esri, Redlands, CA, U.S.A.).

2.3 RESULTS

A total of 423 cattle was sampled from nine dip tanks in June 2016, of which 131 (31.0%) were positive by IgG ELISA. Of 241 cattle tested using SNT, 87 (36.1%) were positive; of these, 75 were IgG ELISA-positive, giving an estimated sensitivity for the IgG ELISA of 86.2% (95% CI: 77.1-92.7%). Of 154 SNT-negative animals, 152 were IgG ELISA-negative, giving an estimated specificity of 98.7% (95% CI: 95.4-99.8%).

2.3.1 Seroprevalence

The seroprevalence in cattle sampled from nine dip tanks in June 2016, based on IgG ELISA and SNT, was 34.0% (144/423; 95% CI 29.5-38.0%). Seroprevalence at dip tanks varied from 18% to 54% and it was high in all age groups (Table 2.1). The multiple logistic regression model (Table 2.1) showed significant differences between dip tanks, with the odds of seropositivity at Mbangwini (Odds ratio (OR) = 4.5; 95% CI: 1.8-11; *P*=0.001), Phelandaba (OR=4.6; 95% CI: 1.8-12; *P*=0.001) and Shemula (OR=5.8; 95% CI: 2.3-15; *P*<0.001) being higher than at Madlakude. Odds of seropositivity was the lowest in the 2-4 y age group, being significantly higher in the 4-6 y age group (OR=2.4; 95% CI: 1.3-4.4; *P*=0.006), tending to be higher in animals >6 y (*P*=0.095), and tending to be higher in females than in males (*P*=0.064). Of the 144 seropositive animals, 114 were tested using the IgM ELISA and 5 (4.4%) were positive; this included 2/6 (33%) of samples that were SNT-positive but IgG ELISA-negative. Therefore, the estimated prevalence of IgM-positive animals in the general population was 1.3% (5/393; 95% CI: 0.4-2.9%).

Table 2.1. Rift Valley fever seroprevalence in cattle at dip tanks in far northern KwaZulu-Natal, June 2016.

Variable	n	Seroprevalence (%)*	95% CI
Diptank			
Madlakude	50	18	7 - 29
Masondo	21	19	2 - 36
Mpala	52	25	13 - 37
Namaneni	50	26	14 - 38
Ndumo	50	30	17 - 43
Hlanjwana	50	34	21 - 47
Phelandaba	50	44	30 - 58
Mbangwini	50	48	34 - 62
Shemula	50	54	40 - 68
Age (years)			
<2	56	34	22 - 46
2 – <4	96	23	15 - 31
4 – 6	157	39	31 - 47
>6	114	34	25 - 43
Sex			
female	331	36	31 - 41
male	92	27	18 - 36
Total	423	34.0	29.5 - 38.0

* Based on IgG ELISA, confirmed by serum neutralization test in 241/423 samples.

The seroprevalence in goats sampled during February - April 2017, determined using SNT, was 31.7% (33/104; 95% CI: 22.9-41.6%) (Table 2.2). Although seroprevalence appeared to increase with age, this was not statistically significant (*P*=0.549) in the multiple logistic regression model (Table 2.2), nor was there a significant difference between sexes (*P*=0.139). The overall seroprevalence in cattle and goats varied substantially across the study area (Fig. 2.3), with foci of high seroprevalence along the Pongolo River, but was not clearly linked to the presence of wetlands.

Table 2.2. Rift Valley fever seroprevalence in goats in far northern KwaZulu-Natal, February-April 2017.

Variable	n	Seroprevalence (%)*	95% CI
Age (years)			
0.5 – 1.5	25	24	9 - 45
>1.5 – 3.5	36	28	14 - 45
>3.5	43	40	25 - 56
Sex			
female	70	36	25 - 48
male	28	18	6 - 37
Total	104	31.7	22.9 - 41.6

* Based on serum neutralization test.

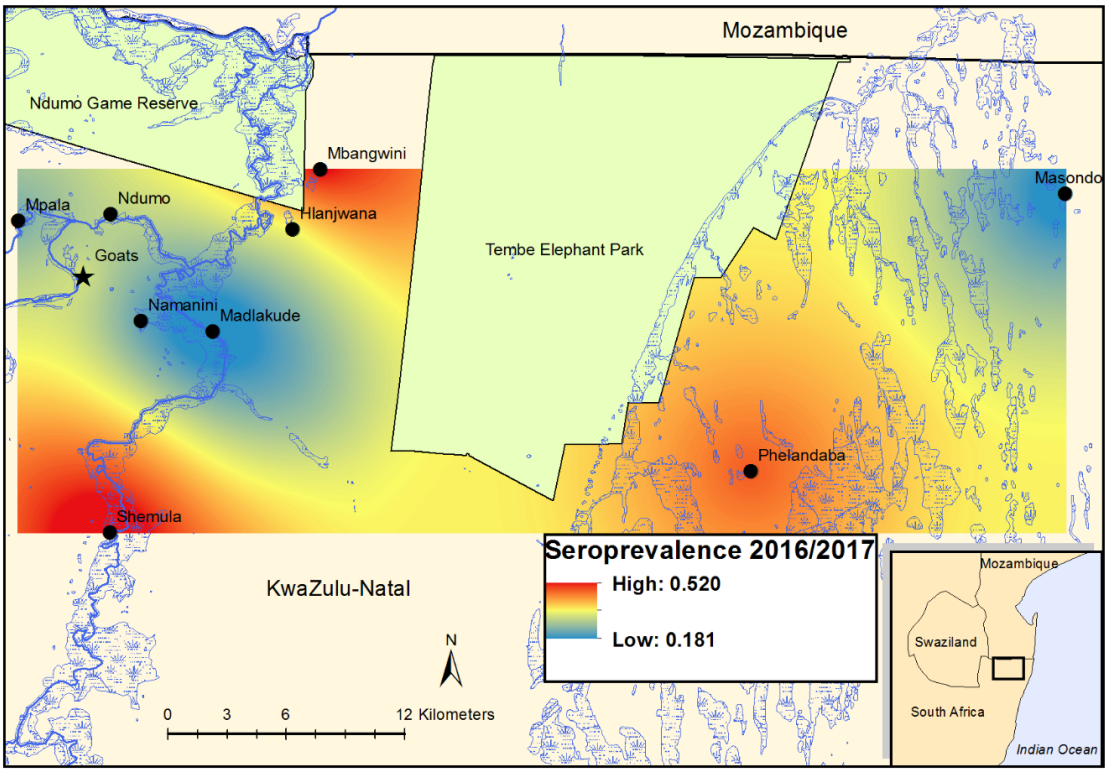


Figure 2.3: Rift Valley fever virus seroprevalence in cattle and goats in far northern KwaZulu-Natal determined using IgG ELISA and serum neutralization test: risk surfaces created by interpolation using ordinary kriging. The map was constructed for this manuscript in Esri® ArcGIS 10.2 using country boundaries from Esri® ArcGIS Online, dip tank coordinates collected during the study, and river (River_FEPAs.shp), wetland (NFEPA_Wetlands.shp) and protected area boundary

(ekznw_pabnd_2015_wdd.zip) data available under a Creative Commons Attribution (CC BY 4.0) license.

2.3.2 Seroconversion rate

Of the 279 cattle that were seronegative in June 2016 by IgG ELISA, or by ELISA and SNT if both tests were used, 103 were re-sampled between one and eight times during the study period. An additional 91 SNT-seronegative cattle were recruited at various times during the study period and re-sampled at least once, therefore 194 initially seronegative animals were sampled at least twice over periods ranging between 28 and 721 days. Assuming that seroconversions occurred, on average, midway between the last negative and first positive test, a total of 111.8 animal-years at risk were observed. Due to various factors, including logistical reasons and varying farmer compliance, the majority of animals (167/194) were followed up at four of the dip tanks (Namaneni, Mpala, Shemula and Ndumo), with very few followed up at Hlanjwana (13), Madlakude (8) and Masondo (6) and none at Mbangwini and Phelandaba.

Seroconversion, determined by SNT, occurred at some stage during the study period in 66 (34%) of initially seronegative cattle. Seroconversions were observed during every interval between samplings for the duration of the study, and at every dip tank followed except Masondo, where only six animals were followed for five months each. The overall incidence rate of seroconversion in cattle between June 2016 and June 2018 was 0.59 seroconversions per animal-year (95% CI: 0.46-0.75) (Table 3). Seroconversion rate varied between dip tanks, with the lowest rate recorded at Namaneni (0.35 per animal-year) and the highest rates observed at Hlanjwana (1.50 per animal-year) and Ndumo (1.01 per animal-year) (Fig. 2.4). There was no clear relationship at dip tank level between the initial seroprevalence in 2016 and the subsequent rate of seroconversion, and the correlation between the two outcomes was not significant (Spearman's ρ=0.29; P=0.535).

Table 2.3. Incidence rate of seroconversion* to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal between June 2016 and June 2018, expressed as numbers of seroconversions per animal-year.

CATTLE	Year 1 (June 2016 – June 2017)			Year 2 (July 2017 – June 2018)			Total		
	Animal-years at risk†	Incidence rate	95% CI	Animal-years at risk†	Incidence rate	95% CI	Animal-years at risk†	Incidence rate	95% CI
Diptank									
Mpala	16.87	0.83	0.45 - 1.39	11.09	0.18	0.02 - 0.65	27.96	0.57	0.33 - 0.93
Ndumo	11.17	1.34	0.75 - 2.21	6.64	0.45	0.09 - 1.32	17.81	1.01	0.60 - 1.60
Namaneni	16.44	0.36	0.13 - 0.79	15.00	0.33	0.11 - 0.78	31.44	0.35	0.17 - 0.63
Shemula	13.74	0.73	0.35 - 1.34	10.64	0.19	0.02 - 0.68	24.37	0.49	0.25 - 0.86
Hlanjwana	3.25	1.84	0.68 - 4.01	0.75	0.00	0.00 - 4.90	4.01	1.50	0.55 - 3.26
Madlakude	3.74	0.80	0.17 - 2.35	0	-	-	3.74	0.80	0.17 - 2.35
Masondo	2.41	0.00	0.00 - 1.53	0	-	-	2.41	0.00	0.00 - 1.53
TOTAL	**67.62**	**0.80**	**0.60 - 1.04**	**44.13**	**0.27**	**0.14 - 0.47**	**111.75**	**0.59**	**0.46 - 0.75**
GOATS	**Year 1 (February – June 2017)**			**Year 2 (July 2017 – June 2018)**			**Total**		
	Animal-years at risk†	Incidence rate	95% CI	Animal-years at risk†	Incidence rate	95% CI	Animal-years at risk†	Incidence rate	95% CI
TOTAL	**14.20**	**0.92**	**0.49 - 1.57**	**34.32**	**0.20**	**0.08 - 0.42**	**48.52**	**0.41**	**0.25 - 0.64**

* Seroconversion defined as a previously seronegative animal becoming seropositive based on the serum neutralization test at a serum dilution of 1:10 or greater.

† Seroconversion assumed to have occurred midway between the last negative and the first positive test.

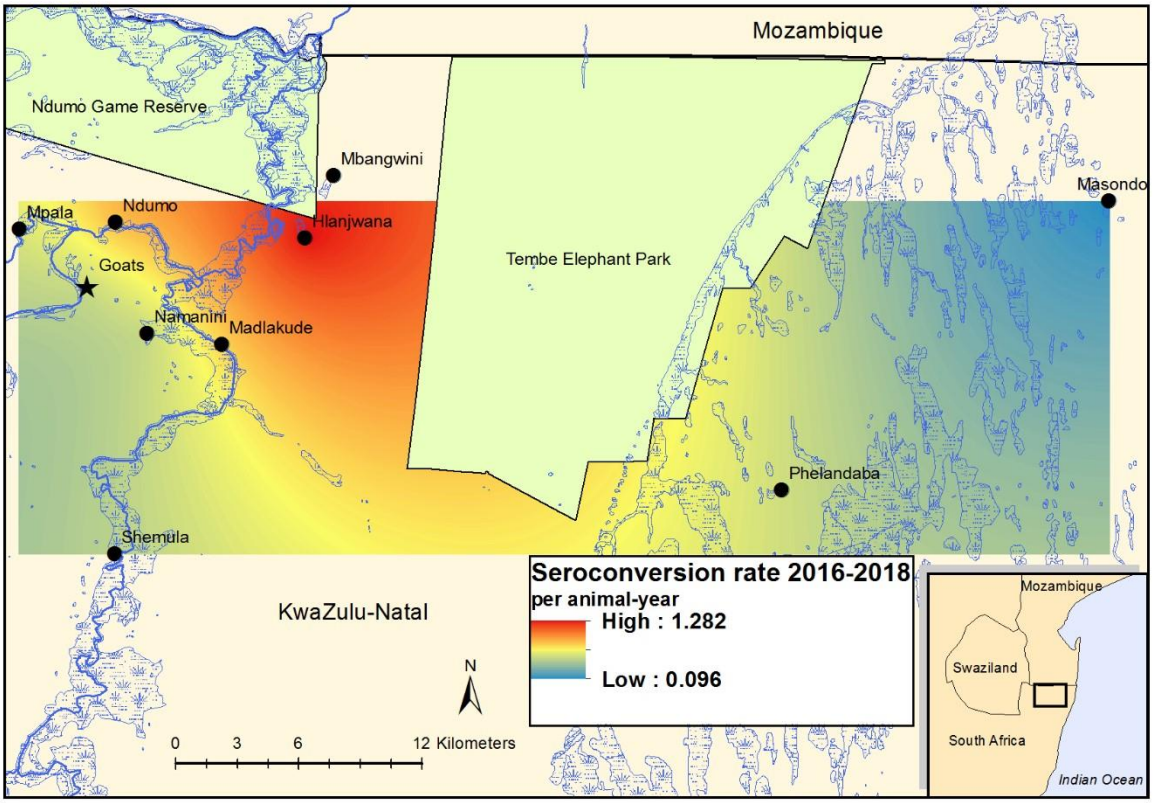


Figure 2.4. Rift Valley fever virus seroconversion rate in cattle and goats in far northern KwaZulu-Natal between June 2016 and June 2018, determined by serum neutralization test: risk surfaces created by interpolation using ordinary kriging. The map was constructed for this manuscript in Esri® ArcGIS 10.2 using country boundaries from Esri® ArcGIS Online, dip tank coordinates collected during the study, and river (River_FEPAs.shp), wetland (NFEPA_Wetlands.shp) and protected area boundary (ekznw_pabnd_2015_wdd.zip) data available under a Creative Commons Attribution (CC BY 4.0) license.

There was a marked variation in the estimated rate of seroconversion in cattle over time, with the rate being lower during the second year of the study (Table 2.3). Seroconversion rate increased from October 2016 to peak at just over 1.5 per animal-year between February and April 2017, dropping to almost zero during August-September 2017 and then showing a lower peak of just over 0.5 per animal-year during December-January 2018 (Fig. 2.5).

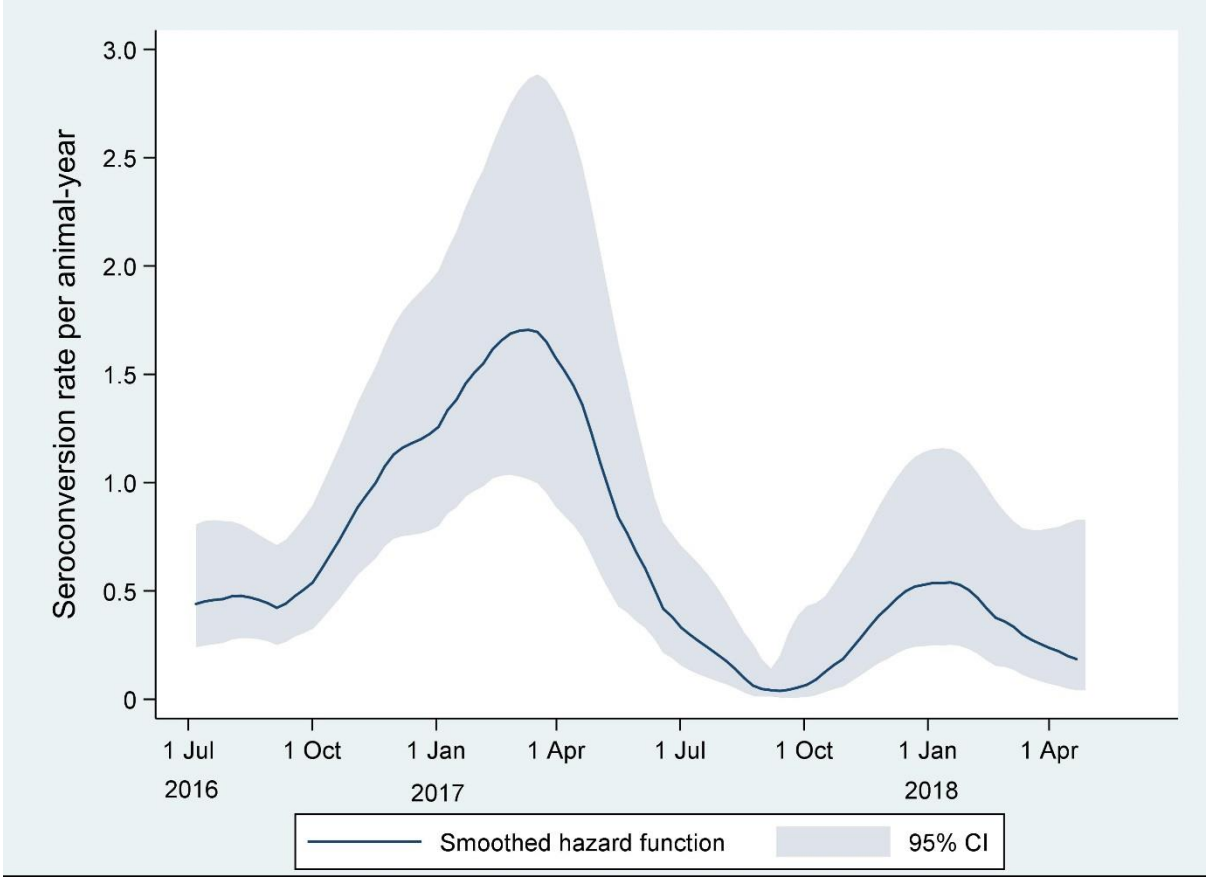


Figure 2.5. Seroconversion rate of cattle to Rift Valley fever virus in far northern KwaZulu-Natal between June 2016 and June 2018, determined by serum neutralization test and expressed as numbers of seroconversions per animal-year, plotted using the derivative of the kernel-smoothed Nelson-Aalen cumulative hazard estimator.

Of the goats that were initially seronegative, 95 were followed up for periods of time ranging between 28 and 482 days, of which 20 (21%) seroconverted. A total of 48.5 animal-years at risk were observed. The overall incidence rate of seroconversion in goats between February 2017 and June 2018 was 0.41 seroconversions per animal-year (95% CI: 0.25-0.64) (Table 2.3) and was also markedly lower during the second summer of the study (Fig. 2.6). The seroconversion rate is not shown after December 2017 since no further seroconversions were observed in the study animals.

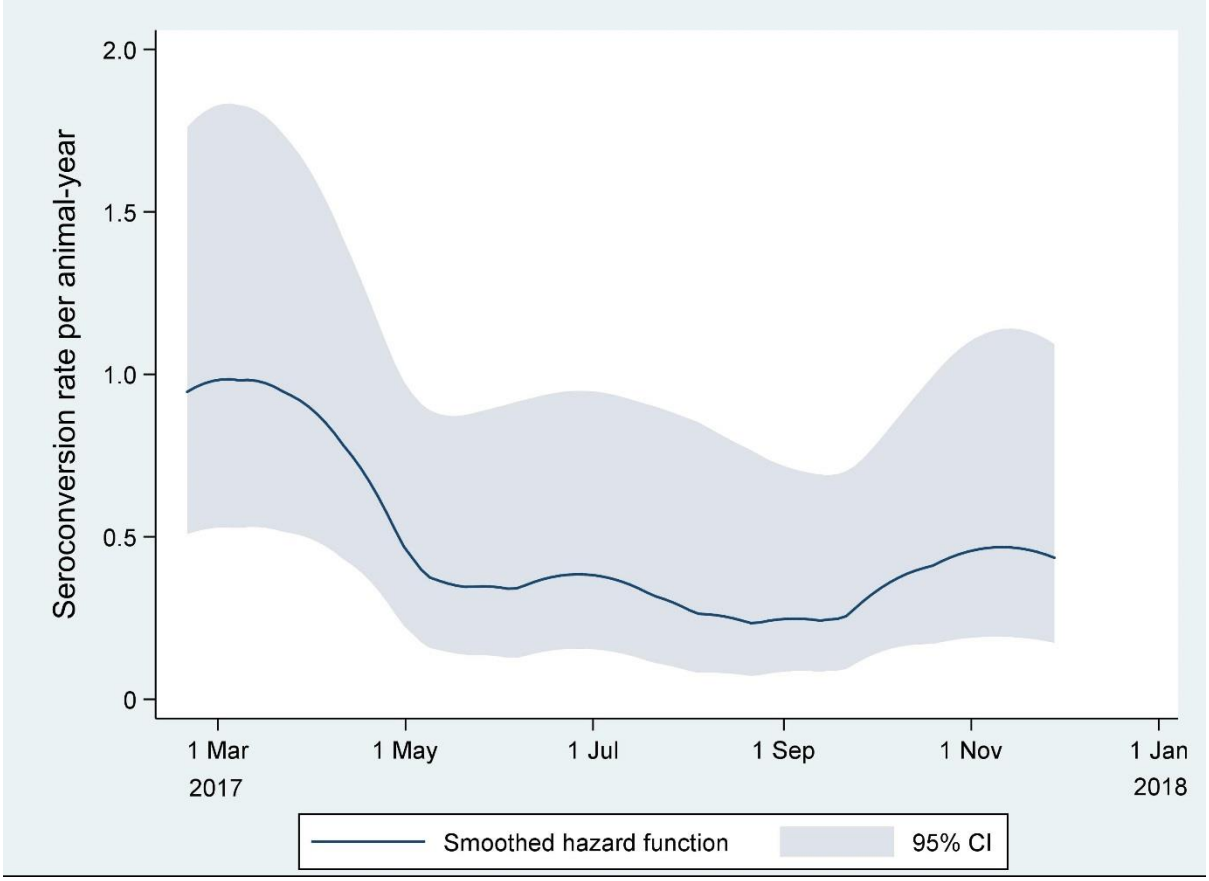


Figure 2.6. Seroconversion rate of goats to Rift Valley fever virus in far northern KwaZulu-Natal between February and December 2017, determined by serum neutralization test and expressed as numbers of seroconversions per animal-year, plotted using the derivative of the kernel-smoothed Nelson-Aalen cumulative hazard estimator.

For cattle and goats combined, the overall rate of seroconversion was 0.54 per animal-year (95% CI: 0.43-0.66) and was 0.82 per animal-year (95% CI: 0.63-1.04) during the first year and 0.24 per animal-year (95% CI: 0.15-0.38) during the second year. The overdispersion parameter α in the negative binomial regression model was not significant (P=0.472), therefore the Poisson model was used. The associations of species, sex, study year and place with seroconversion rate in the multiple Poisson regression model are shown in Table 2.4. The rate did not differ significantly between species and sexes. However, the rate during the second study year was only a third of that during the first year (P<0.001), and significant differences were observed

between locations, with Ndumo and Hlanjwana showing higher rates than Namaneni, where the lowest rate was recorded.

Table 2.4. Factors associated with incidence rate of seroconversion to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal between June 2016 and June 2018.

Variable and level	Incidence rate ratio	95% CI	*P*-value
Species			
Cattle	1*	-	-
Goats	1.62	0.77 - 3.41	0.206
Sex			
Female	1*	-	
Male	0.64	0.36 - 1.14	0.131
Year			
2016-2017	1*	-	-
2017-2018	0.31	0.18 - 0.53	<0.001
Place			
Namanini	1*	-	-
Mpala	1.48	0.69 - 3.19	0.318
Ndumo	2.39	1.11 - 5.13	0.025
Hlanjwana	3.01	1.10 - 8.21	0.031
Madlakude	1.46	0.40 - 5.26	0.566
Shemula	1.40	0.62 - 3.19	0.417
Masondo	0.00	0.00 - ∞	0.977

* Reference level

2.4 DISCUSSION

This study showed evidence of a high level of exposure to RVFV in the study area, demonstrating active circulation of RVFV in livestock not associated with reported outbreaks, and to our knowledge is the first published study to quantify directly the seroconversion rate in livestock using the gold-standard SNT. Previous studies to detect interepidemic circulation have inferred low-level viral transmission in epidemic-prone areas by ELISA-based detection of IgG or IgM.

The study was done in an area of South Africa where outbreaks of RVF have never been reported, although occasional small outbreaks and suspected cases had occurred in the more temperate inland and coastal areas to the south of the study area between 1972 and 1986 (Pienaar and Thompson, 2013, McIntosh *et al*., 1980, McIntosh *et al*., 1983). Elsewhere in Africa, endemic circulation of RVFV is known or suspected to occur in many areas. Limited numbers of RVFV infections were confirmed to occur in sheep and cattle every year during a seven-year interepidemic period in Zimbabwe (Swanepoel, 1981). A seroprevalence of 33% in cattle and 26% in goats was reported during an IEP in outbreak-prone Garissa County, Kenya during 2013, six years after the last major outbreak (Nanyingi *et al*., 2017). Along the Nile River in Sudan, where outbreaks also occur, a prevalence of 33% in cattle and 34% in goats was found (Eisa, 1984). In Mauritania, also in an area that had experienced outbreaks, a seroprevalence of 15% was detected in cattle and 4% in small ruminants, and IgM and viral RNA was detected in one bovine (Rissmann *et al*., 2017). A series of surveys in Mayotte found seroprevalences of up to 37% in cattle in the absence of reported outbreaks (Cêtre-Sossah *et al*., 2012). A seroprevalence of 17% was reported in Rwanda, also in the absence of reported outbreaks (Umuhoza *et al*., 2017). In Maputo Province, Mozambique, neighbouring our study area to the north, a survey in 2010-2011, during the large outbreak in the central interior of South Africa, found a seroprevalence in cattle of 37%, similar to that in our study (Lagerqvist *et al*., 2013). This was followed in 2014 by the detection of a small outbreak of abortion in goats due to RVFV, less than 100 km north of our study area and in the same ecological zone (Fafetine *et al*., 2016). The 1.3% IgM seroprevalence in this study was comparable to that reported in other endemic areas, e.g. 0.5% in Tanzania (Sumaye *et al*., 2013) and

0-4% in Mayotte (Umuhoza *et al*., 2017), indicating the presence of low numbers of recent infections. The seroprevalence in the current study was therefore similar to or higher than those reported elsewhere in Africa where interepidemic circulation is known or suspected to occur and is consistent with ongoing or endemic circulation of RVFV with low numbers of clinical cases likely occurring but overlooked, misdiagnosed or unreported.

Seroprevalence was high in all age groups throughout the study area. Numerous studies have shown that seroprevalence is usually higher in older animals (Fafetine *et al*., 2013, Sumaye *et al*., 2013, Blomström *et al*., 2016, Boussini *et al*., 2014), although in northern Somalia it was higher in younger animals (Soumare *et al*., 2007). The high seroprevalence in young animals can be a result of a recent outbreak; however, it is unlikely that a large, extensive outbreak would have gone unnoticed. In addition, rainfall in the study area during 2014-2016 had been well below average and the study area, as well as much of South Africa had experienced the worst drought in 23 years (Porta, 2014). Release of water from the Pongolapoort Dam had not occurred and most of the floodplain pans were empty. Nevertheless, permanent water was still present in the major rivers and some pans, providing breeding habitat for the mosquito vectors. It is therefore likely that the high seroprevalence in all age groups is indicative of a hyperendemic situation with continuous or sporadic viral activity despite the dry conditions and including recent viral circulation (Porta, 2014).

Rate or force of infection is a key parameter in modelling of infectious diseases in an attempt to understand the factors determining disease transmission. The current study showed a high seroconversion rate to RVFV in livestock, even during dry conditions, with substantial spatial and temporal variation. Low levels of seroconversion in sentinel sheep and goats have recently been reported in Kenya in areas in which outbreaks are known to occur (Lichoti *et al*., 2014, Mbotha *et al*., 2018); however, positive tests were not confirmed using virus neutralization and incidence rates were not reported. In the Kruger National Park (KNP), South Africa, seroconversion was demonstrated in 5/227 African buffalo (*Syncerus caffer*), giving a low annual incidence rate of 1-3% (Beechler *et al*., 2015). This was also demonstrated in the eastern part of the country, adjacent to Mozambique, but outside the tropical zone and in a wildlife area with no

livestock and low human population. The epidemiology of RVFV in our study area more likely approximates that in other tropical areas of Africa and therefore our results provide useful insight into the dynamics of RVFV transmission in endemic areas, both during IEPs in areas where outbreaks are known to occur, and in areas where outbreaks are not reported. Specifically, the range of seroprevalence and seroconversion rates observed in our study will inform the design of further epidemiological studies in known or suspected endemic areas, as well as the modelling of RVFV transmission in such ecosystems. Knowledge of likely force of infection in endemic areas, and its seasonal variation, will also inform studies to assess the risk of spread of RVFV from such areas via animal movements.

As expected, the highest infection rate was seen during mid to late summer, when water levels are highest, and mosquitoes are most abundant. However, despite the fact that rainfall was much higher during the second year of the study, the seroconversion rate was markedly lower, approximately one third of that observed during the first year. It is possible that the drought over the 1-2-year period prior to the study resulted in lower than normal infection rates with a resultant increased number of susceptible animals. This would have increased the number of viraemic animals available to infect mosquitoes, resulting in a higher than usual infection rate during the first year, followed by fewer viraemic animals during the second year. However, the suitable breeding habitat for vectors depends not only on local rainfall, but also on water levels in rivers that are determined by rainfall in their catchment areas. The important mosquito vector species in the study area, and their host preferences, are currently poorly known, and many other factors likely determine their population dynamics, most of which are poorly understood. This study showed very little difference in seroprevalence and seroconversion rate between cattle and goats, suggesting that RVFV is transmitted by one or more mosquito species without strict host preferences.

The differences in seroprevalence and in seroconversion rates between locations could not be readily explained based on geographic location or environmental factors. Two dip tanks with high seroprevalence and seroconversion rates (Mbangwini and Hlanjwana, respectively) were close together and near the lower (northern) Phongolo

River; however, they could not be distinguished from the other locations close to the river based on environmental or climatic variables and separate rainfall figures were not available for each location; animal management practices were also similar for all locations. Therefore, the risk and rate of RVFV infection are likely determined by a complex interplay of host, vector and environmental factors which our results show may vary substantially within a region, even over small distances. This is consistent with the marked spatial variation in seroprevalence observed in other studies (Mbotha *et al*., 2018, Cêtre-Sossah *et al*., 2012, Lagerqvist *et al*., 2013, Boussini *et al*., 2014). The lack of an association between seroprevalence (reflecting past seroconversion rate) and subsequent seroconversion rate in our study, as well as the differences in seroconversion rates between the first and second years of the study, also indicates that these factors vary substantially over time. More detailed, fine-scale eco-epidemiological studies to describe the dynamics of these virus-vector-host-environment interactions are required in order to better understand the behaviour of and risk posed by RVFV in such endemic ecosystems.

The seroprevalence in livestock found in this study was much higher than the 12% observed in 1955, the only previous study done in this region (Kokernot *et al*., 1962). Since then the human and livestock populations in the area have increased tremendously, resulting in a substantial increase in RVFV host density, and it is possible that this has changed the dynamics of viral circulation. Nevertheless, the absence of sheep, the most susceptible livestock species, and the extensive farming system with year-round breeding may be responsible for the fact that large outbreaks do not occur in the area. However, sporadic cases of abortion and neonatal mortality are unlikely to be reported and investigated and may be ascribed to other causes; in addition, disease surveillance in the area is sub-optimal and access to veterinary services is difficult. Therefore, the contribution of RVF to livestock morbidity in the area is unknown and requires further investigation.

Likewise, the potential impact of RVF on human health in the area is unknown. It is generally considered that human infection by RVFV in southern Africa occurs mainly via contact with infected animal tissues from clinical cases and that infection from mosquito bites is less common, as most human cases have a history of exposure to

infected animal tissues and the known mosquito vectors tend to be zoophilic (Swanepoel and Coetzer, 2004). Therefore, the lack of reported cases in livestock in our study area suggests that human exposure to the virus may be low. However, the last published serological surveys done in this region were in the 1950's, in which 16% of human sera (19/118) collected from locations within in our present study area were protective against RVFV (Smithburn *et al*., 1959). To our knowledge there has never been a report of clinical RVF in humans in the area; however, the fact that malaria is common there may result in other causes of febrile illness being overlooked. In support of this hypothesis, a recent study in Maputo, 100 km north of our study area, found a 10% RVFV IgG seroprevalence in febrile patients at a primary health care facility, with seroconversion to RVFV in 5%, concluding that undiagnosed RVFV infections occur in that region and that most RVF cases are misdiagnosed as malaria (Gudo *et al*., 2016). Therefore, the current status and potential impact of RVFV infection in humans in our study area, and in similar areas along the coastal plain of southern Africa, urgently require further investigation.

2.5 CONCLUSION

The results of this study show the presence of RVFV antibodies and active seroconversion in domestic ruminants in far northern KZN, indicating that RVFV is circulating in the area at a rate that varies by location, season and year. Further investigation should be done into the important mosquito vectors in the area, the factors affecting viral circulation and survival, and the impact of the presence of RVFV on animal and human health.

REFERENCES

1. ADAMS, M. J., LEFKOWITZ, E. J., KING, A. M., HARRACH, B., HARRISON, R. L., KNOWLES, N. J., KROPINSKI, A. M., KRUPOVIC, M., KUHN, J. H. & MUSHEGIAN, A. R. 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Archives of Virology,* 162**,** 2505-2538.
2. ARCHER, B. N., THOMAS, J., WEYER, J., CENGIMBO, A., LANDOH, D. E., JACOBS, C., NTULI, S., MODISE, M., MATHONSI, M. & MASHISHI, M. S. 2013. Epidemiologic investigations into outbreaks of Rift Valley fever in humans, South Africa, 2008–2011. *Emerging Infectious Diseases,* 19**,** 1918.
3. BALKHY, H. H. & MEMISH, Z. A. 2003. Rift Valley fever: an uninvited zoonosis in the Arabian peninsula. *International Journal of Antimicrobial Agents,* 21**,** 153-157.
4. BEECHLER, B. R., BENGIS, R., SWANEPOEL, R., PAWESKA, J. T., KEMP, A., VAN VUREN, P. J., JOUBERT, J., EZENWA, V. & JOLLES, A. E. 2015. Rift Valley fever in Kruger National Park: Do buffalo play a role in the inter-epidemic circulation of virus? *Transboundary and Emerging Diseases,* 62**,** 24-32.
5. BENNETT, S., WOODS, T., LIYANAGE, W. M. & SMITH, D. L. 1991. A simplified general method for cluster-sample surveys of health in developing countries. *World Health Stat Q*. 1991;44(3):98-106. PMID: 1949887.
6. BLOMSTRÖM, A.-L., SCHARIN, I., STENBERG, H., FIGUEIREDO, J., NHAMBIRRE, O., ABILIO, A., BERG, M. & FAFETINE, J. 2016. Seroprevalence of Rift Valley fever virus in sheep and goats in Zambézia, Mozambique. *Infection Ecology and Epidemiology,* 6**,** 31343.
7. BOUSSINI, H., LAMIEN, C., NACOULMA, O., KABORÉ, A., PODA, G. & VILJOEN, G. 2014. Prevalence of Rift Valley fever in domestic ruminants in the central and northern regions of Burkina Faso. *Revue Scienstifique et Technique,* 33**,** 893-901.
8. BRITCH, S. C., BINEPAL, Y. S., RUDER, M. G., KARIITHI, H. M., LINTHICUM, K. J., ANYAMBA, A., SMALL, J. L., TUCKER, C. J., ATEYA, L. O. & ORIKO, A. A. 2013. Rift Valley fever risk map model and seroprevalence in selected wild ungulates and camels from Kenya. *PLoS One,* 8**,** e66626.
9. BRUTON MN. Introduction. In: Bruton MN, Cooper KH, editors. Studies on the ecology of Maputoland. Grahamstown: Rhodes University; 1980. p. xvii-xix.
10. CÊTRE-SOSSAH, C., PÉDARRIEU, A., GUIS, H., DEFERNEZ, C., BOULOY, M., FAVRE, J., GIRARD, S., CARDINALE, E. & ALBINA, E. 2012. Prevalence of Rift Valley fever among ruminants, Mayotte. *Emerging Infectious Diseases,* 18**,** 972.
11. DAUBNEY, R., HUDSON, J. & GARNHAM, P. 1931. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep cattle and man from East Africa. *The Journal of Pathology and Bacteriology,* 34**,** 545-579.
12. EISA, M. 1984. Preliminary survey of domestic animals of the Sudan for precipitating antibodies to Rift Valley fever virus. *Epidemiology & Infection,* 93**,** 629-637.
13. EZEMVELO PROTECTED AREA BOUNDARY (ekznw_pabnd_2015_wdd.zip) [Internet]. Biodiversity Conservation Planning Division, Ezemvelo KZN Wildlife; c2015 [cited 2018 Oct 29]. Available from: http://www.sasdi.net/metaview.aspx?uuid=78e859b1c3161984b3e02739c58b3241.
14. FAFETINE, J., NEVES, L., THOMPSON, P. N., PAWESKA, J. T., RUTTEN, V. P. & COETZER, J. A. 2013. Serological evidence of Rift Valley fever virus circulation in sheep and goats in Zambezia Province, Mozambique. *PLoS Neglected Tropical Diseases,* 7**,** e2065.

15. FAFETINE, J. M., COETZEE, P., MUBEMBA, B., NHAMBIRRE, O., NEVES, L., COETZER, J. A. & VENTER, E. H. 2016. Rift Valley fever outbreak in livestock, Mozambique, 2014. *Emerging Infectious Diseases,* 22**,** 2165.
16. FAGBO, S., COETZER, J. A. & VENTER, E. H. 2014. Seroprevalence of Rift Valley fever and lumpy skin disease in African buffalo (*Syncerus caffer*) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa. *Journal of the South African Veterinary Association,* 85**,** 01-07.
17. GLANCEY, M. M., ANYAMBA, A. & LINTHICUM, K. J. 2015. Epidemiologic and environmental risk factors of Rift Valley Fever in southern Africa from 2008 to 2011. *Vector-Borne and Zoonotic Diseases,* 15**,** 502-511.
18. GUDO, E. S., PINTO, G., WEYER, J., LE ROUX, C., MANDLAZE, A., JOSÉ, A. F. & PAWESKA, J. T. 2016. Serological evidence of Rift Valley fever virus among acute febrile patients in Southern Mozambique during and after the 2013 heavy rainfall and flooding: implication for the management of febrile illness. *Virology Journal,* 13**,** 96.
19. JOZINI LOCAL MUNICIPALITY INTEGRATED DEVELOPMENT PLAN 2017/18–2021/22 [Internet]. Jozini Municipality; c2017 [cited 2018 Sep 16]. Available from: www.jozini.gov.za/phocadownload/idpbudget/June2017/jozini%20municipality%2017_18%20%20idp.pdf.
20. JUPP, P., GROBBELAAR, A., LEMAN, P., KEMP, A., DUNTON, R., BURKOT, T., KSIAZEK, T. & SWANEPOEL, R. 2000. Experimental detection of Rift Valley fever virus by reverse transcription-polymerase chain reaction assay in large samples of mosquitoes. *Journal of Medical Entomology,* 37**,** 467-471.
21. KARBER, G. 1931. 50% end point calculation. *Archiv fur Experimentelle Pathologie und Pharmakologie,* 162**,** 480-483.
22. KOKERNOT, R., HEYMANN, C., MUSPRATT, J. & WOLSTENHOLME, B. 1957. Studies on arthropod-borne viruses of Tongaland. V. Isolation of Bunyamwera and Rift Valley Fever viruses from mosquitoes. *South African Journal of Medical Sciences,* 22**,** 71-80.
23. KOKERNOT, R., MCINTOSH, B., WORTH, C. B., DE MORAIS, T. & WEINBREN, M. 1962. Isolation of Viruses from Mosquitoes Collected at Lumbo, Mozambique. *The American Journal of Tropical Medicine and Hygiene,* 11**,** 678-682.
24. KSIAZEK, T., JOUAN, A., MEEGAN, J., LE GUENNO, B., WILSON, M., PETERS, C., DIGOUTTE, J., GUILLAUD, M., MERZOUG, N. O. & TOURAY, E. 1989. Rift Valley fever among domestic animals in the recent West African outbreak. *Research in Virology,* 140**,** 67-77.
25. LABEAUD, A. D. 2008. Why arboviruses can be neglected tropical diseases. *PLoS Neglected Tropical Diseases,* 2**,** e247.
26. LAGERQVIST, N., MOIANE, B., MAPACO, L., FAFETINE, J., VENE, S. & FALK, K. I. 2013. Antibodies against Rift Valley fever virus in cattle, Mozambique. *Emerging Infectious Diseases,* 19**,** 1177.
27. LAUGHLIN, L. W., MEEGAN, J. M., STRAÜSBAUGH, L., MORENS, D. M. & WATTEN, R. H. 1979. Epidemic Rift Valley fever in Egypt: observations of the spectrum of human illness. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73**,** 630-633.
28. LICHOTI, J. K., KIHARA, A., ORIKO, A. A., OKUTOYI, L. A., WAUNA, J. O., TCHOUASSI, D. P., TIGOI, C. C., KEMP, S., SANG, R. & MBABU, R. M. 2014. Detection of Rift Valley fever virus interepidemic activity in some hotspot areas of Kenya by sentinel animal surveillance, 2009–2012. *Veterinary Medicine International,* 2014.
29. LINTHICUM, K., DAVIES, F., KAIRO, A. & BAILEY, C. 1985. Rift Valley fever virus (family *Bunyaviridae*, genus *Phlebovirus*). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Epidemiology & Infection,* 95**,** 197-209.

30. MBOTHA, D., BETT, B., KAIRU-WANYOIKE, S., GRACE, D., KIHARA, A., WAINAINA, M., HOPPENHEIT, A., CLAUSEN, P. H. & LINDAHL, J. 2018. Inter-epidemic Rift Valley fever virus seroconversions in an irrigation scheme in Bura, south-east Kenya. *Transboundary and Emerging Diseases,* 65**,** e55-e62.
31. MCINTOSH, A., JUPP, P. & DOS SANTOS, I. 1983. Field and laboratory evidence implicating *Culex zombaensis* and *Aedes circumluteolus* as vectors of Rift Valley fever virus in coastal South Africa. *South African Journal of Science,* 79**,** 61-64.
32. MCINTOSH, B. 1972. Rift valley fever: 1. Vector studies in the field. *Journal of the South African Veterinary Association,* 43**,** 391-395.
33. MCINTOSH, B., JUPP, P., DOS SANTOS, I. & BARNARD, B. 1980. Vector studies on Rift Valley fever virus in South Africa. *South African Medical Journal,* 58**,** 127-32.
34. MEEGAN, J. M. 1979. The Rift Valley fever epizootic in Egypt 1977–1978 1. Description of the epizootic and virological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73**,** 618-623.
35. MOIANE, B., MAPACO, L., THOMPSON, P., BERG, M., ALBIHN, A. & FAFETINE, J. 2017. High seroprevalence of Rift Valley fever phlebovirus in domestic ruminants and African buffaloes in Mozambique shows need for intensified surveillance. *Infection Ecology and Epidemiology,* 7**,** 1416248.
36. MOLL, E. 1980. Terrestrial plant ecology. *Studies on the Ecology of Maputaland***,** 52-68.
37. MORVAN, J., SALUZZO, J.-F., FONTENILLE, D., ROLLIN, P. & COULANGES, P. 1991. Rift Valley fever on the east coast of Madagascar. *Research in Virology,* 142**,** 475-482.
38. MIURA, Y., KUBO, M., GOTO, Y. & KONO, Y. 1991. Chuzan disease as congenital-hydranencephaly cerebellar hypoplasia syndrome in calves. *Japan Agricultural Research Quarterly*, 25:55-60
39. NANYINGI, M. O., MUCHEMI, G. M., THUMBI, S. M., ADE, F., ONYANGO, C. O., KIAMA, S. G. & BETT, B. 2017. Seroepidemiological survey of Rift Valley fever virus in ruminants in Garissa, Kenya. *Vector-Borne and Zoonotic Diseases,* 17**,** 141-146.
40. NIKLASSON, B., LILJESTRAND, J., BERGSTRÖM, S. & PETERS, C. 1987. Rift Valley fever: a sero-epidemiological survey among pregnant women in Mozambique. *Epidemiology and Infection,* 99**,** 517-522.
41. OLALEYE, O., TOMORI, O. & SCHMITZ, H. 1996. Rift Valley fever in Nigeria: infections in domestic animals. *Revue scientifique et technique-Office international des épizooties,* 15**,** 937-946.
42. PAWESKA, J. T., BURT, F. J., ANTHONY, F., SMITH, S. J., GROBBELAAR, A. A., CROFT, J. E., KSIAZEK, T. G. & SWANEPOEL, R. 2003. IgG-sandwich and IgM-capture enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in domestic ruminants. *Journal of Virological methods,* 113**,** 103-112.
43. PIENAAR, N. J. & THOMPSON, P. N. 2013. Temporal and spatial history of Rift Valley fever in South Africa: 1950 to 2011. *Onderstepoort Journal of Veterinary Research,* 80**,** 1-13.
44. PORTA, M. 2014. *A dictionary of epidemiology*, Oxford university press.
45. RISSMANN, M., EIDEN, M., EL MAMY, B., ISSELMOU, K., DOUMBIA, B., ZIEGLER, U., HOMEIER-BACHMANN, T., YAHYA, B. & GROSCHUP, M. 2017. Serological and genomic evidence of Rift Valley fever virus during inter-epidemic periods in Mauritania. *Epidemiology and Infection,* 145**,** 1058-1068.
46. River Freshwater Ecosystem Priority Areas (FEPAs) (River_FEPAs.shp) [Internet]. South African National Biodiversity Institute; c2016 [cited 2018 May 30]. Available from: www.sasdi.net/metaview.aspx?uuid=0dadbc5046456801420ebea919939f99.
47. ROGERS, K. 1980. The vegetation of the Pongolo floodplain: distribution and utilization. *Studies of the Eecology of Maputaland***,** 69-77.

48. ROMOSER, W. S., OVIEDO, M. N., LERDTHUSNEE, K., PATRICAN, L. A., TURELL, M. J., DOHM, D. J., LINTHICUM, K. J. & BAILEY, C. L. 2011. Rift Valley fever virus-infected mosquito ova and associated pathology: possible implications for endemic maintenance. *Research and Reports in Tropical Medicine,* 2**,** 121.
49. ROSTAL, M. K., EVANS, A. L., SANG, R., GIKUNDI, S., WAKHULE, L., MUNYUA, P., MACHARIA, J., FEIKIN, D. R., BREIMAN, R. F. & NJENGA, M. K. 2010. Identification of potential vectors of and detection of antibodies against Rift Valley fever virus in livestock during interepizootic periods. *American Journal of Veterinary Research,* 71**,** 522-526.
50. SHOEMAKER, T., BOULIANNE, C., VINCENT, M. J., PEZZANITE, L., AL-QAHTANI, M. M., AL-MAZROU, Y., KHAN, A. S., ROLLIN, P. E., SWANEPOEL, R. & KSIAZEK, T. G. 2002. Genetic analysis of viruses associated with emergence of Rift Valley fever in Saudi Arabia and Yemen, 2000-01. *Emerging Infectious Diseases,* 8**,** 1415.
51. SISSOKO, D., GIRY, C., GABRIE, P., TARANTOLA, A., PETTINELLI, F., COLLET, L., D'ORTENZIO, E., RENAULT, P. & PIERRE, V. 2009. Rift valley fever, Mayotte, 2007–2008. *Emerging Infectious Diseases,* 15**,** 568.
52. SMITHBURN, K., HEYMANN, C., WEINBREIN, M. & ZENTKOWSKY, D. 1959. Neutralizing antibodies for certain viruses in the sera of human beings residing in northern Natal. *South African Medical Journal,* 33.
53. SOUMARE, B., TEMPIA, S., CAGNOLATI, V., MOHAMOUD, A., VAN HUYLENBROECK, G. & BERKVENS, D. 2007. Screening for Rift Valley fever infection in northern Somalia: a GIS based survey method to overcome the lack of sampling frame. *Veterinary Microbiology,* 121**,** 249-256.
54. SUMAYE, R. D., GEUBBELS, E., MBEYELA, E. & BERKVENS, D. 2013. Inter-epidemic transmission of Rift Valley fever in livestock in the Kilombero River Valley, Tanzania: a cross-sectional survey. *PLoS Neglected Tropical Diseases,* 7**,** e2356.
55. SWANEPOEL, R. Observations on Rift Valley fever in Zimbabwe. Proceedings... Rift Valley fever; a workshop, 1981.
56. SWANEPOEL, R. & COETZER, J. 2004. Rift valley fever. *Infectious Diseases of Divestock,* 2**,** 1037-1070.
57. THRUSFIELD, M. 2018. *Veterinary Epidemiology*, 3rd ed. Oxford: Blackwell Science; 2007.
58. UMUHOZA, T., BERKVENS, D., GAFARASI, I., RUKELIBUGA, J., MUSHONGA, B. & BIRYOMUMAISHO, S. 2017. Seroprevalence of Rift Valley fever in cattle along the Akagera-Nyabarongo rivers, Rwanda. *Journal of the South African Veterinary Association,* 88**,** 1-5.
59. VOGEL, C. AND VAN ZYL, K., 2016. Drought: In search of sustainable solutions to a persistent,'wicked'problem in South Africa. In Climate Change Adaptation Strategies–An Upstream-downstream Perspective (pp. 195-211). Springer, Cham.
60. WETLAND FRESHWATER ECOSYSTEM PRIORITY AREAS (NFEPA_Wetlands.shp) [Internet]. South African National Biodiversity Institute; c2016 [cited 2018 May 30]. Available from: www.sasdi.net/metaview.aspx?uuid=330feba934dbd53fc832cee55768e188.
61. WORLD ORGANISATION FOR ANIMAL HEALTH (OIE). Rift Valley fever. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 7th ed. Paris: OIE; 2012. ch. 2.1.14.

Chapter 3

Neutralizing antibodies against Rift Valley fever virus in wild antelope in far northern KwaZulu-Natal, South Africa, indicate recent viral circulation

This chapter was submitted to *Transboundary and Emerging Diseases* for publication.

Abstract

Rift Valley fever is a zoonotic viral disease of domestic ruminants in Africa and the Arabian Peninsula caused by a mosquito-borne *Phlebovirus*. Outbreaks in livestock and humans occur after heavy rains that favour breeding of the vectors, and the virus is thought to survive dry seasons in the eggs of floodwater-breeding aedine mosquitoes. We recently found high seroconversion rates to RVFV in cattle and goats in the absence of outbreaks in far northern KZN, South Africa, and here report the prevalence of, and factors associated with, neutralizing antibodies to RVFV in 326 sera from nyala (*Tragelaphus angasii*) and impala (*Aepyceros melampus*) culled during 2016-18 in two nature reserves in the same area. The overall seroprevalence of RVFV was 35.0% (114/326; 95% CI 29.8-40.4%) and tended to be higher in Ndumo Game Reserve (11/20; 55% 95% CI 31.5-76.9%) than in Tembe Elephant Park (103/306; 33.6%; 95% CI 28.4-39.3%) (P = 0.087). The presence of antibodies in juveniles (6/21; 28.6%; 95% CI 11.3-52.2%) and sub-adults (13/65; 20.0%; 95% CI 11.1-37.8%) confirmed that infections had occurred subsequent to the 2008-2011 RVF outbreaks in South Africa. Seroprevalence was highest in adults and odds of seroprevalence was inversely associated with distance from a swamp or floodplain. Under similar ecological conditions, domestic and wild ruminants may play a similar role in maintenance of viral circulation and either or both may serve as the mammalian host in a vector-host reservoir system. The study confirms the high rate of recent circulation of RVFV in the tropical coastal plain of northern KZN and provides the basis

for further investigation of factors affecting viral circulation and the role of wildlife in the epidemiology of RVF.

3.1 INTRODUCTION

Rift Valley fever is a zoonotic mosquito-borne disease of ruminants in Africa and the Arabian Peninsula caused by RVFV within the genus *Phlebovirus,* family *Phenuiviridae* (Adams *et al*., 2017, Swanepoel and Coetzer, 2004). Outbreaks of RVFV are recognized by abortion storms in domestic ruminants and deaths of young animals. Humans can become infected from contact with infected tissues of livestock, or less frequently form mosquito bites, and usually undergo benign febrile illness, but may develop fatal haemorrhagic fever, encephalitis, or ocular sequelae (Wilson, 1994). The virus was discovered in Kenya in 1930 (Daubney *et al*., 1931), and the disease was first recognized in South Africa in a major epidemic in 1950-51 (Gear *et al*., 1951), with further large-scale outbreaks occurring in 1974-76 (Barnard, 1977) and 2008-11 (Metras *et al*., 2012). Large outbreaks usually occur after exceptionally heavy rains that favour breeding of the mosquito vectors; in southern Africa, such circumstances tend to follow La Niña events in the Pacific Ocean (Anyamba *et al*., 2001).

The virus is believed to overwinter through transovarial passage in certain species of floodwater-breeding Aedine mosquitoes (Linthicum *et al*., 1985). Their eggs need to undergo a degree of desiccation before infected adult mosquitoes emerge following floods. The infection is then transmitted to susceptible animals that in turn serve as a source of virus for susceptible mosquitoes when taking viraemic blood meals (Swanepoel and Coetzer, 2004). When heavy rains result in a population explosion of mosquitoes, larger numbers of animals become infected, and culicine mosquitoes that breed in standing water then also serve as epidemic vectors to intensify the outbreaks (Linthicum *et al*., 1983).

During the first recognized outbreak in South Africa in 1951, abortions were observed to occur in antelope, including springbok and blesbok, although RVFV was not proven

to be the cause (Alexander, 1951, Gear *et al*., 1951). In 1999 abortions in a waterbuck and six African buffaloes were confirmed to be caused by RVFV (Olive *et al*., 2012). Unspecified clinical disease, including death, due to RVFV was reported in African buffalo, springbok, kudu, nyala, sable, roan, gemsbok, blesbok, bontebok and waterbuck during the 2010-2011 RVF outbreaks in the interior of South Africa, although in extremely low numbers compared to the large numbers of domestic ruminants affected (Pienaar and Thompson, 2013, DAFF, 2011).

Antibodies to RVFV have been detected in multiple wildlife species in Africa, including African buffalo, black rhino (*Diceros bicornis*), African elephant (*Loxodonta africana*) and several antelope (Bovidae) species (Evans *et al*., 2008, Beechler *et al*., 2015, Jori *et al*., 2015, Dondona *et al*., 2016, Bird *et al*., 2008). A study conducted in wildlife reserves in Kenya tested sera for RVFV antibodies from 16 species of wildlife; those with the highest seroprevalences included African buffalo (37/237; 16%), black rhino (14/43; 23%), Thomson's gazelle (*Eudorcas thomsonii*) (7/8; 87%), kudu (5/10; 50%), impala (*Aepyceros melampus*) (5/8; 62%) and waterbuck (2/10; 20%) (Evans *et al*., 2008). Although these sera were collected during an inter-epidemic period, many of the animals may have been infected during the previous epidemic; some evidence, however, of inter-epidemic circulation in wildlife was found.

A study using samples collected in 2003-2004 in South Africa found a prevalence of antibodies to RVFV in African buffalo in the KNP of 6.5% and 4.5% in Hluhluwe-iMfolozi Park, KZN, using a RVFV IgG ELISA (Fagbo *et al*., 2014). In the KNP a low rate of seroconversion was reported among buffaloes (9/126; 7%) over a six-year period (2000-2006) using a haemagglutination-inhibition titration assay (LaBeaud *et al*., 2011). Also, in the KNP, 5/227 seronegative female buffaloes seroconverted to RVFV over a 5-year period (2008-2012) (Beechler *et al*., 2015), confirming a very low level of circulation in the absence of observed outbreaks.

It has been suggested that wildlife may serve as RVFV maintenance hosts during inter- epidemic periods, since areas rich in water sources and intermittent wetlands, along with Bovidae species are positively associated with RVFV outbreaks (Walsh *et al*., 2017). The potential role of wildlife in the epidemiology of RVF has been reviewed (Olive *et al*. 2012), and it was concluded that there is no definitive evidence for a

wildlife maintenance host. However, due to the absence of a known carrier state, the maintenance “host” must by necessity be a host-vector system rather than a single species. In the KNP where very low-level seroconversion was found, it has been concluded in this study that a combination of mammalian hosts and vertical transmission by mosquitoes is necessary for RVFV persistence (Manore and Beechler, 2015).

We recently found high seroconversion rates to RVFV in cattle and goats in far northern KZN Province, South Africa, in the absence of reported outbreaks of disease (van den Bergh *et al*., 2019). The livestock tested in that study were in an area adjoining two nature reserves, although separated from wildlife by fences. In order to investigate the potential role of wildlife in RVFV circulation in the area, the objective of this study was to determine the seroprevalence and associated risk factors of RVFV in antelope in the Tembe Elephant Park and the Ndumo Game Reserve, using sera from animals routinely culled over a two-year period.

3.2 MATERIALS AND METHODS

3.2.1 Ethical approval

The study was approved by the Animal Ethics Committee of the University of Pretoria (V013-16) and adhered to the specifications of the South African National Standard (SANS 10386-2008): “The Care and Use of Animals for Scientific Purposes”. Support for the project was obtained from Ezemvelo KZN Wildlife, the KZN conservation authority that manages the two reserves. The project was approved by the Department of Agriculture, Forestry and Fisheries, Republic of South Africa, and the KZN Department of Agriculture and Rural Development (KZNDARD), which issued permits for the movement of animal samples from the foot-and-mouth disease-controlled area to the University of Pretoria.

3.2.2 Study area

The study was conducted in the ~30,000 ha TEP and the ~10,000 ha NGR on the Maputaland coastal plain of northern KZN, which is bordered by the Lebombo Mountains to the west and the Indian Ocean to the east. The Tembe Elephant Park and surrounding areas are covered by open woodlands with grasslands, palmveld and patches of sand forest (Moll and White, 1978, Granger, 1996, Wilson, 1994, Matthews *et al*., 2001). The Muzi swamp that crosses the eastern side of the reserve with its reed beds constitutes the only permanent source of water in the park. The Ndumo Game Reserve, known for its diversity of bird life and large floodplain systems, is situated at the confluence of the Pongolo River and the Usuthu River, which forms the northern boundary of the reserve. The reserve is characterized by diverse habitats of riverine forest, floodplains, grasslands, reed beds, broad-leaved and acacia woodlands, and dense thornveld (Pooley, 1982, Calverley and Downs, 2014). Both reserves are bordered Mozambique to the north, with the TEP border being fenced across woodland and sand forest habitats and the NGR border consisting of the Usuthu River. Both reserves are fenced off from the surrounding livestock areas, where communal subsistence farming is practised. With the exception of the eastern

border of NGR, the fences are well maintained and effective at preventing wildlife-livestock contact; with the TEP fence electrified to contain the elephants.

3.2.3 Sampling and laboratory testing

Serum samples were collected opportunistically during routine culling of nyala and impala antelope for population control purposes in both reserves between June 2016 and May 2018. Animals were harvested by park management at night using a spotlight and rifle and blood was collected into plain Vacutainer® tubes during exsanguination. Serum was separated by centrifugation in a field laboratory at TEP and stored at -20°C for transport to Pretoria under a KZNDARD permit. On arrival, sera were inactivated at 56°C for 1 hour in a water bath to minimize the risk of foot-and-mouth disease virus contamination and stored at -20°C until used. The serum neutralization test was used and is a gold standard for RVFV serodiagnosis (OIE, 2012). The serum neutralization test was performed in 96-well plate (AEC-Amersham) format according to the standard protocol of the Virology Section, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria which follows the method prescribed by the World Organization for Animal Health (OIE, 2012). Briefly: Sera were diluted 1:5 in PBS+ (phosphate buffered saline with added $MgCl_2$ and $CaCl_2$) and two-fold dilutions of the serum were made. The $TCID_{50}$ was determined using the Karber method (Karber, 1931). A volume of 100$TCID_{50}$ virus (Smithburn vaccine strain) was added to each dilution and incubated at 37°C for 60 min. A total of 80 µl of African green monkey kidney cells (Vero) (480,000 cells/ml) in MEM containing 5% foetal calf serum (Biowest, Celtic) was added to each well. The microplates were incubated at 37°C in an atmosphere containing 5% CO_2 and observed daily for cytopathic effect. The titre was calculated as the dilution at which 50% of the cells were affected. Results were only accepted if all controls gave the expected results (virus control, positive serum and negative serum). A serum dilution of ≥1:10 was used to define seropositivity.

3.2.4 Statistical analysis

The period prevalence of seropositivity to RVFV in antelope sera was calculated, overall and by sampling site, species, sex, age group (juvenile, sub-adult, adult), collection year (June 2016 to May 2017 vs. June 2017 to June 2018) and proximity to a floodplain or swamp (<2 km vs. >2 km). Univariable associations were assessed using Fisher's exact test. Locations of sample collection sites, water sources (NFEPA, 2016) and reserve boundaries (Ezemvelo Protected Area Boundary) were plotted in ArcGIS 10.2 (Esri Corporation, Redlands, CA, U.S.A.) and distance to water sources calculated. A multivariable logistic regression model was used to estimate the association of site, species, sex, age, year and proximity to water with the chances of seropositivity to RVFV while controlling for confounding. All two-way interactions were also tested for significance. The fit of the model was assessed using a Hosmer-Lemeshow goodness-of-fit test. Statistical analyses were done using Stata 15 (StataCorp, College Station, TX, U.S.A.) and significance was assessed at $P < 0.05$.

3.3 RESULTS

A total of 326 serum samples were obtained at irregular intervals from June 2016 to June 2018; most were from TEP, with only 20 from NGR, all during August-December 2016 (Fig. 3.1). The seroprevalence over the collection period was 35.0% (114/326; 95% CI: 29.8 - 40.4%) (Table 3.1) and, although somewhat higher for NGR, did not differ significantly between the reserves ($P = 0.087$). There was also no significant difference in seroprevalence between nyala (97/289; 33.5%; 95% CI: 21.0 - 33.0%) and impala (17/37; 45.9%; 95% CI: 28.1 - 39.3%) ($P = 0.146$).

After adjustment for confounding, the multivariable model (Table 3.2) showed that animals that were sampled within 2 km of a floodplain or swamp were more likely to be seropositive (OR = 3.30; 95% CI: 1.70, 6.38; $P < 0.001$) compared to that were > 2km away. This pattern is visible in the distribution of positive and negative samples in TEP but is not clear for NGR (Fig. 3.1). Odds of seropositivity was the lowest in sub-adults and was significantly higher both in juveniles (OR = 4.73; 95% CI: 1.30-17.3; *P*

= 0.019) and in adults (OR = 3.98; 95%CI: 1.83-8.67; *P* = 0.001). Males were more likely than females to be seropositive (OR = 2.66; 95%CI: 1.51, 4.68; *P* = 0.001). No two-way interactions between predictors were significant and were therefore not included in the model. The Hosmer-Lemeshow goodness-of-fit statistic indicated adequate model fit (*P* = 0.528). Restricting the analysis to samples from TEP only, resulted in no material change in the effects of the other predictors.

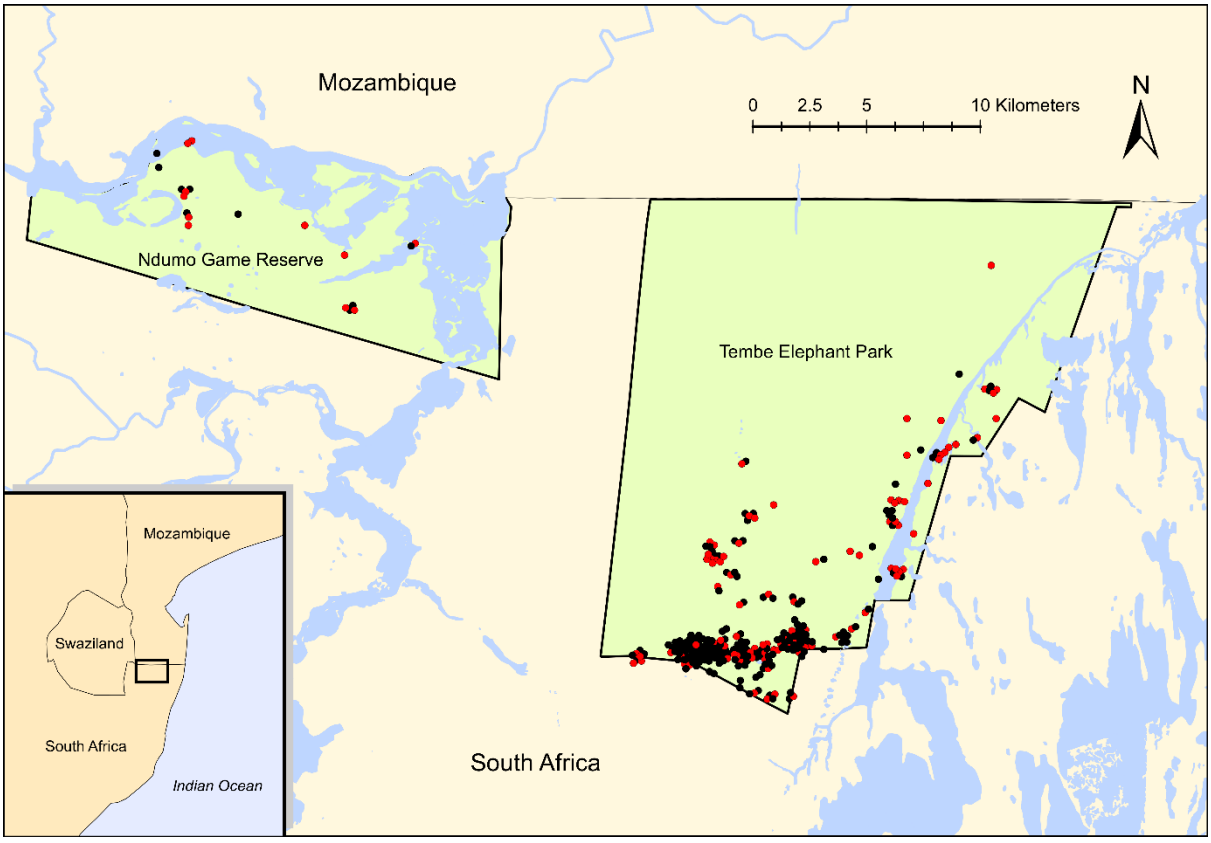


Figure 3.1: A map of Ndumo Game Reserve and Tembe Elephant Park, showing rivers, the maximum extent of floodplains and swamps, and the locations where animals were sampled. Red dots represent Rift Valley fever virus antibody-positive animals and black dots represent seronegative animals.

Table 3.1: Seroprevalence of Rift Valley fever virus in wild antelope in far northern KwaZulu-Natal: descriptive statistics and univariate associations.

Variable	N	Seroprevalence (%)	95% CI (%)	*P*-value
Sampling site				0.087
NGR	20	55.0	31.5 - 76.9	
TEP	306	33.6	28.4 - 39.3	
Sampling year				<0.001
Year 1 (2016 - 2017)	105	52.4	42.4 - 62.2	
Year 2 (2017 - 2018)	221	26.7	21.0 - 33.0	
Species				0.146
Nyala	289	33.6	28.1 - 39.3	
Impala	37	45.9	29.5 - 63.1	
Sex				0.003
Female	172	27.3	20.8 - 34.6	
Male	154	43.5	35.5 - 51.7	
Age				0.009
Juvenile	21	28.6	11.3 - 52.2	
Sub-adult	65	20.0	11.1 - 31.8	
Adult	240	39.5	33.4 - 46.1	
Proximity to floodplain				<0.001
>2km	77	55.8	44.1 - 67.2	
≤ 2km	240	39.6	33.4 - 46.1	
Total	1416	36.3	27.7 - 44.8	

Table 3.2: Factors associated with seropositivity to Rift Valley fever virus in wild antelope in far northern KwaZulu-Natal: multivariable logistic regression model.

Variable and level	Odds ratio	95% CI	*P*-value
Sampling site			
Ndumo	1*	–	–
Tembe	1.65	0.52 - 5.22	0.396
Sampling year			
Year 1 (2016 - 2017)	1*	–	–
Year 2 (2017 - 2018)	0.40	0.22 - 0.72	0.002
Species			
Nyala	1*	–	–
Impala	1.08	0.48 - 2.40	0.859
Sex			
Female	1	–	–
Male	2.66	1.51 - 4.68	0.001
Age			
Juvenile	4.73	1.30 - 17.3	0.019
Sub-adult	1*	–	–
Adult	3.98	1.83 - 8.67	0.001
Proximity to floodplain			
>2 km	1*	–	–
<2 km	3.30	1.70 - 6.38	<0.001

* Reference level

3.4 DISCUSSION

This study reports the seroprevalence of antibodies to RVFV in wild antelope species in NGR and TEP, two reserves adjacent to livestock farming areas in which a high rate of RVFV circulation has recently been reported (van den Bergh *et al*., 2019). The overall seroprevalence in nyala and impala was fairly similar to that in cattle and goats. This pattern was also evident in Kenya, where the seroprevalence of RVFV in wildlife increased in parallel with domestic animals during a major outbreak of disease in livestock, and similarly declined afterwards (Britch *et al*., 2013). Livestock in our study area may graze along the fences of the reserves but, with the exception of a portion of the eastern fence of NGR, they are not able to enter the reserves. Direct transmission from animal to animal is only possible when animals can lick each other or aborted foetuses (Pepin *et al*., 2010). The most likely explanation for the apparently similar seroprevalence in both domestic and wild ruminant populations is that they are all part of the same vector-host maintenance system, including one or more mosquito species without strict host preferences. The definition of a reservoir depends on specifying the target population (Haydon *et al*., 2002); therefore, it is possible that the wildlife-vector system may act as a reservoir for livestock, or the livestock-vector system may act as a reservoir for wild ruminants. In terms of the potential health risk to humans, all three components (livestock, wildlife and vector) may constitute the reservoir, with livestock likely being the most important source population for human infection; although in our study area, where clinical cases are not reported, this remains to be determined.

Antibodies to RVFV have been detected in multiple wildlife species in Africa and the Middle East. In South Africa low level circulation among buffaloes has been recorded during IEPs (LaBeaud *et al*., 2011, Beechler *et al*., 2015) and it has been suggested that wildlife may play an important role in the survival cycle of the virus during these periods (Olive *et al*., 2012). A number of ungulates have been recorded as susceptible to clinical disease due to RVFV (Evans *et al*., 2008, Olive *et al*., 2012, Pienaar and Thompson, 2013, DAFF, 2011), but no further investigation has been done on the transmission efficiency of ungulates or other wildlife. It is also unknown whether RVF causes any disease or has any detrimental effect on wildlife in the area; the only

previously reported occurrence of RVF in nyala was death of a single animal during the outbreak in 2010 on a farm in the Northern Cape, well outside the species' natural range (DAFF, 2011). However, any sporadic clinical cases, particularly abortions, that may occur in wildlife in the study area would likely be overlooked or remain undiagnosed.

Silent circulation of the virus in livestock has been described in adjacent Mozambique, where only a few outbreaks have been reported despite widespread serological evidence of exposure to RVFV in livestock and African buffalo (Fafetine *et al*., 2013, Moiane *et al*., 2017). With recent evidence of active RVFV circulation in livestock (van den Bergh *et al*., 2019) and wild antelope species (this study) in northern KZN, it is evident that the virus may circulate for long periods on the tropical coastal plain of south-eastern Africa with few or no reported outbreaks or diagnosis of clinical cases in humans or animals. In contrast to the KNP, where a low seroprevalence and low seroconversion rate was found in African buffaloes (Fagbo *et al*., 2014, Beechler *et al*., 2015, LaBeaud *et al*., 2011) and it was concluded that a combination of horizontal and vertical (transovarial) transmission by mosquitoes was necessary for RVFV maintenance (Manore and Beechler, 2015), a high seroprevalence was found in this study. Therefore, the role of, and necessity for, transovarial transmission by mosquitoes in the maintenance of RVFV circulation in such tropical lowland areas requires further investigation.

In this study the seroprevalence between the two years was different, with the first year being higher than the second. Seroprevalence may be expected to change over time if the rate of seroconversion changes, which was shown to be the case in the livestock in the adjacent farming areas; the rate of change of seroprevalence depends also on the rate of sero-reversion (Muench, 1959) which is unknown. However, it is difficult to know whether the apparent difference between years reflected a real change in seroprevalence, since animals were sampled by convenience and by different people over time, likely using different criteria for selection. The apparent difference in seroprevalence between the two reserves was not significant in the multivariable model and was likely an artefact due to the small sample size from NGR

and due to confounding, since all the samples from NGR were collected during the first year.

There was a clear and significant positive association between RVFV seroprevalence and proximity of sampling site to surface water, namely the Muzi swamp in TEP. A cut-off of 2 km was selected, using the approximate average maximum distance (2214 m) that *Culex (Cux.) tritaeniorhynchus* would fly in order to find a blood meal (Verdonschot and Besse-Lototskaya, 2014). This was found to be the most abundant mosquito species caught during an entomological study in the same area (unpublished data) and has been shown to be a competent RVFV vector elsewhere (Jupp *et al*., 2002). Similar observations were made in Mayotte where people and animals were more likely to have antibodies to RVFV when they were located near a water source (Lernout *et al*., 2013), likely due to more frequent exposure to vectors.

It is noteworthy that males of both species were more likely to be seropositive than females. Impala males tend to have a larger home range than females (Vincent, 1979); however, this is not the case for nyala. Males of both species, but particularly nyala, are also much larger, and seroprevalence is reported to increase with size in cattle (Jeanmaire *et al*., 2011). It has also been observed in primates that larger animals are more likely to be infected with malaria, presumably due to their higher production rate of chemical attractants (Davies *et al*., 1991). Apart from carbon dioxide, these include kairomones and volatile organic compounds such as ketones and acetones that accumulate in blood of ruminants after feeding, and that are used by haemotophagous diptera to detect the target when searching for a bloodmeal (Clements, 1999). The much larger volume of air exhaled by male antelope such as nyala may therefore significantly increase their attractiveness to mosquitoes. Other factors may be increased thermal radiation and differences in visual stimulation (Clements, 1999) due to the difference in size and colour between male and female nyala; however, further research would be required to confirm this for nyala.

It is unclear why seroprevalence was higher in juveniles than in sub-adults, although the low number of juveniles sampled, and their non-random selection may have reduced the accuracy of the estimate. For most infectious diseases, with a constant rate of exposure, it is expected that there will be a gradual increase in seroprevalence

with increasing age, whereas variations in rate of exposure, which is expected with RVFV, will distort this relationship. Another factor that could have influenced this result is possible incorrect age classification between juveniles and sub-adults during sample collection. Combining juveniles and sub-adults into a single category resulted in a clear difference in seroprevalence between young animals (22%) and adults (40%) which was significant in a multivariable model (OR = 2.65; *P* = 0.003). This increase in seroprevalence with age, along with the fact that the seroprevalence was high in all age groups, indicates that RVFV is endemic at high levels in the wildlife population and that wild antelope may be an important component of the RVFV maintenance system in the study area.

3.5 CONCLUSION

This study demonstrated that circulation of RVFV occurs in antelope in the absence of apparent clinical disease in northern KZN, South Africa, alongside circulation of virus in livestock in adjacent areas. It appears that, under similar ecological conditions, domestic and wild ruminants may play a similar role in maintenance of viral circulation, and either or both may serve as the mammalian host in a vector-host maintenance system. However, very little is known about transmission efficiency and susceptibility of wildlife hosts, or the role of transovarial transmission by mosquitoes. The identity and population dynamics of the important vectors and the impact of the presence of the virus on animal and human health should also be further investigated.

REFERENCES

1. ADAMS, M. J., LEFKOWITZ, E. J., KING, A. M., HARRACH, B., HARRISON, R. L., KNOWLES, N. J., KROPINSKI, A. M., KRUPOVIC, M., KUHN, J. H. & MUSHEGIAN, A. R. 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Archives of Virology,* 162**,** 2505-2538.
2. ALEXANDER, R. 1951. Rift Valley fever in the Union. *Journal of the South African Veterinary Association,* 22**,** 105-112.
3. ANYAMBA, A., LINTHICUM, K. J. & TUCKER, C. J. 2001. Climate-disease connections: Rift Valley fever in Kenya. *Cadernos de Saude Publica,* 17**,** S133-S140.
4. BARNARD, M. 1977. An inactivated rift valley fever vaccine. *Journal of the South African Veterinary Association,* 48**,** 45-48.
5. BEECHLER, B. R., BENGIS, R., SWANEPOEL, R., PAWESKA, J. T., KEMP, A., VAN VUREN, P. J., JOUBERT, J., EZENWA, V. & JOLLES, A. E. 2015. Rift Valley fever in Kruger National Park: Do buffalo play a role in the inter-epidemic circulation of virus? *Transboundary and Emerging Diseases,* 62**,** 24-32.
6. BIRD, B. H., BAWIEC, D. A., KSIAZEK, T. G., SHOEMAKER, T. R. & NICHOL, S. T. 2007. Highly sensitive and broadly reactive quantitative reverse transcription-PCR assay for high-throughput detection of Rift Valley fever virus. *Journal of Clinical Microbiology,* 45**,** 3506-3513.
7. BIRD, B. H., GITHINJI, J. W., MACHARIA, J. M., KASIITI, J. L., MURIITHI, R. M., GACHERU, S. G., MUSAA, J. O., TOWNER, J. S., REEDER, S. A. & OLIVER, J. B. 2008. Multiple virus lineages sharing recent common ancestry were associated with a large Rift Valley fever outbreak among livestock in Kenya during 2006-2007. *Journal of Virology,* 82**,** 11152-11166.
8. BRITCH, S. C., BINEPAL, Y. S., RUDER, M. G., KARIITHI, H. M., LINTHICUM, K. J., ANYAMBA, A., SMALL, J. L., TUCKER, C. J., ATEYA, L. O. & ORIKO, A. A. 2013. Rift Valley fever risk map model and seroprevalence in selected wild ungulates and camels from Kenya. *PLoS One,* 8**,** e66626.
9. CALVERLEY, P. M. & DOWNS, C. T. 2014. Population Status of Nile Crocodiles in Ndumo Game Reserve, Kwazulu-Natal, South Africa (1971–2012). *Herpetologica,* 70**,** 417-425.
10. CLEMENTS, A. N. 1999. *The biology of mosquitoes. Volume 2: sensory reception and behaviour*, CABI Publishing.
11. DAFF 2011. Department of Agriculture, Forestry and Fisheries (DAFF) Disease Database. Retrieved from www.nda.agric.za/vetweb/epidemiology/Disease%20Database/OIEData/OIE_queryCriteria.asp.
12. DAUBNEY, R., HUDSON, J. & GARNHAM, P. 1931. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep cattle and man from East Africa. *The Journal of Pathology and Bacteriology,* 34**,** 545-579.
13. DAVIES, C., AYRES, J., DYE, C. & DEANE, L. 1991. Malaria infection rate of Amazonian primates increases with body weight and group size. *Functional Ecology***,** 655-662.
14. DONDONA, A. C., ASCHENBORN, O., PINONI, C., DI GIALLEONARDO, L., MASEKE, A., BORTONE, G., POLCI, A., SCACCHIA, M., MOLINI, U. & MONACO, F. 2016. Rift valley fever virus among wild ruminants, Etosha National Park, Namibia, 2011. *Emerging Infectious Diseases,* 22**,** 128.
15. EISA, M. 1984. Preliminary survey of domestic animals of the Sudan for precipitating antibodies to Rift Valley fever virus. *Epidemiology & Infection,* 93**,** 629-637.

16. EKZN Wildlife. (2015). Ezemvelo Protected Area boundary (ekznw_pabnd_2015_wdd.zip). Biodiversity Conservation Planning Division, Ezemvelo KZN Wildlife. Retrieved from www.sasdi.net/metaview.aspx?uuid=78e859b1c3161984b3e02739c58b3241.
17. EVANS, A., GAKUYA, F., PAWESKA, J., ROSTAL, M., AKOOLO, L., VAN VUREN, P. J., MANYIBE, T., MACHARIA, J., KSIAZEK, T. & FEIKIN, D. 2008. Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife. *Epidemiology & Infection,* 136**,** 1261-1269.
18. FAFETINE, J., NEVES, L., THOMPSON, P. N., PAWESKA, J. T., RUTTEN, V. P. & COETZER, J. A. 2013. Serological evidence of Rift Valley fever virus circulation in sheep and goats in Zambezia Province, Mozambique. *PLoS Neglected Tropical Diseases,* 7**,** e2065.
19. FAGBO, S., COETZER, J. A. & VENTER, E. H. 2014. Seroprevalence of Rift Valley fever and lumpy skin disease in African buffalo (Syncerus caffer) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa. *Journal of the South African Veterinary Association,* 85**,** 01-07.
20. GEAR, J., DE MEILLO, B., LE ROUX, A., KOFSKY, R., ROSE, R., STEYN, J., OLIFF, W. & SCHULZ, K. 1955. Rift valley fever in South Africa: A study of the 1953 outbreak in the Orange Free State, with special reference: To the vectors and possible reservoir hosts. *South African Medical Journal,* 29.
21. GEAR, J., DE MEILLON, B., MEASROCH, V. & DAVIS, D. 1951. Rift Valley fever in South Africa: 2: the occurrence of human cases in the Orange Free State, the North-Western Cape Province, the Western And Southern Transvaal: B: field and laboratory investigations. *African Journal of Health Professions Education,* 25**,** 908-912.
22. JEANMAIRE, E. M., RABENARIVAHINY, R., BIARMANN, M., RABIBISOA, L., RAVAOMANANA, F., RANDRIAMPARANY, T., FY ANDRIAMANDIMBY, S., DIAW, C. S., FENOZARA, P. & DE LA ROCQUE, S. 2011. Prevalence of Rift Valley fever infection in ruminants in Madagascar after the 2008 outbreak. *Vector-Borne and Zoonotic Diseases,* 11**,** 395-402.
23. JORI, F., ALEXANDER, K. A., MOKOPASETSO, M., MUNSTERMANN, S., MOAGABO, K. & PAWESKA, J. T. 2015. Serological evidence of Rift Valley fever virus circulation in domestic cattle and African buffalo in Northern Botswana (2010–2011). *Frontiers in Veterinary Science,* 2**,** 63.
24. JUPP, P., KEMP, A., GROBBELAAR, A., LEMAN, P., BURT, F., ALAHMED, A., MUJALLI, D. A., KHAMEES, M. A. & SWANEPOEL, R. 2002. The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Medical and Veterinary Entomology,* 16**,** 245-252.
25. KARBER, G. 1931. 50% end point calculation. *Archiv fur Experimentelle Pathologie und Pharmakologie,* 162**,** 480-483.
26. LABEAUD, A. D., CROSS, P. C., GETZ, W. M., GLINKA, A. & KING, C. H. 2011. Rift Valley fever virus infection in African buffalo (Syncerus caffer) herds in rural South Africa: evidence of interepidemic transmission. *The American Journal of Tropical Medicine and Hygiene,* 84**,** 641-646.
27. LERNOUT, T., CARDINALE, E., JEGO, M., DESPRÈS, P., COLLET, L., ZUMBO, B., TILLARD, E., GIRARD, S. & FILLEUL, L. 2013. Rift valley fever in humans and animals in Mayotte, an endemic situation? *PLoS One,* 8**,** e74192.
28. LINTHICUM, K., DAVIES, F., BAILEY, C. & KAIRO, A. 1983. Mosquito species succession in a dambo in an East African forest [Kenya]. *Mosquito News (USA)*.
29. LINTHICUM, K., DAVIES, F., KAIRO, A. & BAILEY, C. 1985. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Epidemiology & Infection,* 95**,** 197-209.

30. MANORE, C. & BEECHLER, B. 2015. Inter-epidemic and between-season persistence of Rift Valley fever: Vertical transmission or cryptic cycling? *Transboundary and Emerging Diseases,* 62**,** 13-23.
31. MATTHEWS, W., VAN WYK, A., VAN ROOYEN, N. & BOTHA, G. 2001. Vegetation of the Tembe Elephant Park, Maputaland, South Africa. *South African Journal of Botany,* 67**,** 573-594.
32. METRAS, R., PORPHYRE, T., PFEIFFER, D. U., KEMP, A., THOMPSON, P. N., COLLINS, L. M. & WHITE, R. G. 2012. Exploratory space-time analyses of Rift Valley fever in South Africa in 2008–2011. *PLoS Neglected Tropical Diseases,* 6**,** e1808.
33. MOIANE, B., MAPACO, L., THOMPSON, P., BERG, M., ALBIHN, A. & FAFETINE, J. 2017. High seroprevalence of Rift Valley fever phlebovirus in domestic ruminants and African buffaloes in Mozambique shows need for intensified surveillance. *Infection ecology & epidemiology,* 7**,** 1416248.
34. MOLL, E. & WHITE, F. 1978. The Indian Ocean coastal belt. *Biogeography and ecology of southern Africa.* Dordrecht: Springer.
35. MUENCH, H. 1959. *Catalytic models in epidemiology*, Harvard University Press Cambridge, MA.
36. NFEPA. 2016. *Wetland Freshwater Ecosystem Priority Areas (NFEPA_Wetlands.shp) [Internet]. South African National Biodiversity Institute;* [Online]. Available: www.sasdi.net/metaview.aspx?uuid=330feba934dbd53fc832cee55768e188. [Accessed].
37. OIE 2012. World Organisation for Animal Health. Rift Valley fever. In*: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (7th ed.). Paris.
38. OLIVE, M.-M., GOODMAN, S. M. & REYNES, J.-M. 2012. The role of wild mammals in the maintenance of Rift Valley fever virus. *Journal of Wildlife Diseases,* 48**,** 241-266.
39. PEPIN, M., BOULOY, M., BIRD, B. H., KEMP, A. & PAWESKA, J. 2010. Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Veterinary Research,* 41**,** 61.
40. PIENAAR, N. J. & THOMPSON, P. N. 2013. Temporal and spatial history of Rift Valley fever in South Africa: 1950 to 2011. *Onderstepoort Journal of Veterinary Research,* 80**,** 1-13.
41. POOLEY, A. C. 1982. The ecology of the Nile crocodile Crocodylus niloticus in Zululand. MSc thesis. University of Natal.
42. SWANEPOEL, R. & COETZER, J. 2004. Rift valley fever. *Infectious Diseases of Livestock,* 2**,** 1037-1070.
43. VAN DEN BERGH, C., VENTER, E. H., SWANEPOEL, R. & THOMPSON, P. N. 2019. High seroconversion rate to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal, South Africa, in the absence of reported outbreaks. *PLOS Neglected Tropical Diseases,* 13**,** e0007296.
44. VERDONSCHOT, P. F. & BESSE-LOTOTSKAYA, A. A. 2014. Flight distance of mosquitoes (Culicidae): a metadata analysis to support the management of barrier zones around rewetted and newly constructed wetlands. *Limnologica-Ecology and Management of Inland Waters,* 45**,** 69-79.
45. VINCENT, J. 1979. *The population dynamics of impala in the Mkuze Game reserve, Zululand.* Ph. D. thesis, University of Natal.
46. WALSH, M. G., DE SMALEN, A. W. & MOR, S. M. 2017. Wetlands, wild Bovidae species richness and sheep density delineate risk of Rift Valley fever outbreaks in the African continent and Arabian Peninsula. *PLoS Neglected Tropical Diseases,* 11**,** e0005756.
47. WILSON, M. L. 1994. Rift Valley Fever Virus Ecology and the Epidemiology of Disease Emergence a. *Annals of the New York Academy of Sciences,* 740**,** 169-180.

Chapter 4

Mosquito population dynamics in an area of Rift Valley fever virus circulation in northern KwaZulu-Natal, South Africa

This chapter is written in preparation for publication – (therefore the repetition of general facts).

Abstract

Longitudinal mosquito surveys were carried out in the northern part of KZN, South Africa from November 2016 to June 2018, in a sub-tropical area known for its river floodplains. The study aim was to investigate the diversity, abundance, and seasonal dynamics of mosquitoes, comparing three different sites, and to screen mosquitoes for RVFV. Monthly collections of adult mosquitoes were carried out using CO_2-baited tent traps. Mosquitoes were identified, pooled (n≤50) and screened for RVFV by real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) directed toward amplification of a 217-bp fragment of the L segment of the virus. A total of 34,848 mosquitoes (Diptera: Culicidae), belonging to 7 genera and 48 species, were captured. *Culex* (*Cux.*) *tritaeniorhynchus* (31%), *Cx.* (*Cux.*) *antennatus* (29%), *Aedes* (*Adm.*) *durbanensis* (12%) and *Cx.* (*Cux.*) *neavei* (10%) were the most abundant species collected. The presence of different genera was noted between the collection sites. Cumulative rainfall and average minimum temperature for 30 days prior to collection were positively associated with the number of mosquitoes collected while average maximum temperature was only associated with the number of *Culex* spp. caught. A single pool of *Ae. durbanensis* was found to be positive for RVFV genomic RNA.

4.1 INTRODUCTION

Rift Valley fever virus (Family *Phenuiviridae*, genus *Phlebovirus*) (Adams *et al*., 2017) causes severe outbreaks in domestic ruminants in sub-Saharan Africa, Africa and the Middle East (Pepin *et al*., 2010). Currently it poses a threat to countries outside of

Africa, such as Europe and the Americas (Rolin *et al*., 2013). The disease predominantly causes abortion storms in domestic ruminants and is responsible for fatal infection in newborn animals, but humans and wildlife can also be infected (Meegan and Shope, 1981, Evans *et al*., 2008).

The occurrence of RVF outbreaks is initiated by increased and prolonged rainfall (Davies *et al*., 1985). Conditions predisposing to outbreaks have been defined as flooding of temporary wetlands (pans or dambos) in areas where RVFV circulates (Linthicum *et al*., 1985). This flooding is followed by the hatching of large numbers of floodwater *Aedes* mosquitoes. There is evidence that RVFV is carried over to the next generation of *Aedes* mosquitoes via eggs that are deposited in seasonal pans that dry up during low rainfall seasons. The eggs can withstand extended periods of drought (Linthicum *et al*., 1985). *Aedes* mosquitoes that have been identified to play the largest role in transmission of RVFV in South Africa include *Aedes (Och.) caballus/juppi* and *Ae. (Neo.) mcintoshi* on the inland plateau, and *Ae. (Neo.) circumluteolus* in KZN (Jupp, 1996). *Culex* species such as *Cx. (Cux.) theileri*, *Cx. (Cux.) zombaensis* and *Cx. (Cux.) quinquefasciatus* have been incriminated as the vectors that transmit the virus during epizootic periods on the plateau of Gauteng, Mpumalanga and Free State provinces (Jupp, 1996, McIntosh *et al*., 1980).

It has been shown that endemic circulation can take place in areas in South Africa and Mozambique without clinical signs being detected. RVFV was responsible for a small outbreak in goats in the Maputo Province, close to the South African border, in 2014 (Fafetine *et al*., 2016). Recently, the overall seroprevalence of RVFV antibodies in seven provinces of Mozambique was reported to be 37% in cattle, 30% in African buffalo (*Syncerus caffer*), and 29% in domestic ruminants in the Maputo Province (Moiane *et al*., 2017). In far northern KZN, a parallel study to this one (Chapter 2) showed the presence of RVFV and active seroconversion in domestic ruminants. Furthermore, antibodies to RVFV were found in nyala (*Tragelaphus angasii*) and impala (*Aepyceros melampus*) in two nature reserves, Ndumo Game Reserve and Tembe Elephant Park (Chapter 3).

During a small outbreak in 1981 in the coastal region of KZN, it was concluded that *Cx. zombaensis* and *Ae. circumluteolus* were the main vectors. About 120 cattle were

infected during this outbreak with eleven reported abortions (McIntosh *et al*., 1983). A vector study was done in Port Shepstone on the southern KZN coast during 1969-1971 during which RVFV was isolated from *Eretmapodites quinquevittatus*; other mosquitoes caught were *Anopheles (Ano.) coustani* and *Ae. (Ste.) strelitziae* (McIntosh, 1972). The most common species collected in Mozambique not far from the Natal border in 1960-68 (McIntosh *et al*., 1972) included *Ae. durbanensis, Ae. circumluteolus, Cx. (Cux.) thalassius, Cx. (Cux.) poicilipes* and *An. (Ano.) coustani*. In another study during the same time period in KZN, *Ae. (Neo.) circumluteolus* dominated (Kokernot *et al*., 1957a). It is known that *Ae. (Neo.) circumluteolus* is more common in the coastal zone of northern KZN where RVFV has been isolated twice in 1955 (McIntosh, 1972). *Aedes (Neo.) circumluteolus* was the most abundant mosquito species caught on the shores of Lake Simbu in northern KZN and it was hypothesized that it might be the primary vector of RVFV in the area. *Mansonia (Man.) uniformis* was less common while *Cx. poicilipes* was the most common species collected among humans described in the study (Kokernot *et al*., 1957a). In a later study attempts were made to isolate RVFV from 400,000 mosquitoes collected in the same areas of northern KZN but were not successful (McIntosh *et al*., 1983). From these studies it is clear that little is known about the range of species responsible for the maintenance and transmission of RVFV in South Africa, particularly in the eastern coastal area.

The aim of the present study was to investigate the diversity, abundance, and seasonal dynamics of mosquitoes collected in northern KZN. Results from three localities were compared and mosquitoes were screened for the presence of RVFV.

4.2 MATERIALS AND METHODS

4.2.1 Study area

The study was conducted on the Maputaland coastal plain in far northern KZN just south of the Mozambique border, an area with a sub-tropical climate characterised by warm, dry winters and hot, wet summers. In the Pongolo River floodplain, the core of the study area, the summer temperatures range from 23-40°C and winter temperatures range from 16-26°C. The region experiences summer rainfall from November to mid-April with the mean annual rainfall approximately 600-800 mm (Jozini local municipality integrated development plan 2017/18 – 2021/22). The northwards flowing Pongolo River forms a floodplain at an elevation of 30-50 m above sea level, approximately 10-15 km to the east of the foothills of the Lebombo Mountains, which rise to about 650 m above sea level. The study area contains a mosaic of bushland, thicket, wooded grassland, riverine forest and floodplain vegetation (Moll, 1980). Depending on rainfall, seasonal flooding of the Pongolo, Ingwavuma and Usuthu Rivers inundates about 13,000 ha of floodplain, filling numerous pans, some of which retain water during the dry season (Rogers, 1980). The construction of the Pongolapoort Dam in 1973 largely disrupted this cycle, with flooding only occurring with particularly heavy rainfalls or with periodic opening of sluice gates (Rossouw, 1985). In very dry years, permanent water may only be present in the Pongolo and Usuthu Rivers and in very few of the larger pans. The present study started during a drought period when most of the pans were dry. The collection period was from November 2016 to June 2018, averaging 2-3 nights per month.

4.2.2 Collection sites

Tent traps were placed at three locations in northern KZN. The first site (Mpala) was 10-20 m from the water edge at an inlet of a small stream into the Qotho pan, on the floodplain of the Ingwavuma River about 5 km from its confluence with the Pongolo River (Fig. 4.1). The pan was usually dry, but the water inlet had some water in it for most of the study period, except during the late winter months to spring (July to

September 2017). The water inlet was used for to supply water to green pepper crops that were planted by local farmers about 50 m from the collection site. Cattle grazed around the pan but there were no human settlements in the immediate vicinity. A dip tank was located about 150 m from the collection site where cattle were dipped weekly as part of a foot-and-mouth disease surveillance programme. The collection site was about 4 km from the Ndumo Game Reserve border.

Figure 4.1: Mosquito trapping at Mpala collection site during summer

The second collection site was at the perimeter of Bumbe pan which forms part of the Pongolo river floodplain (referred to as Bumbe area) (Fig. 4.2). The collection site was surrounded by fever trees (*Vachellia xanthophloea*) and no crops were planted around the collection sites during the study. This might be because the pan was dry for most of the period, although the Pongola river about 0.5 km from the collection site constituted a permanent water source for mosquito breeding. The pan had some water during March and April. The collection site was 10.9 km from the Mpala collection site. Cattle grazed in close proximity to the site throughout the study period.

Figure 4.2: Mosquito trapping at Bumbe collection site during summer

The third location was added to the study from October 2017 and was located about 1.4 km from Bumbe pan. This location was different from the other locations in that there were human settlements around the pan. The collection location was about 4 m from the water edge next to Makhana pan (Fig. 4.3). The pan had water for most of the study period. The location was 10 km from Ndumo Game Reserve.

Figure 4.3: Mosquito trapping at Makhana collection site on a summer night

The locations of the collection sites in context with rivers, floodplains and each other are illustrated in Fig. 4.4.

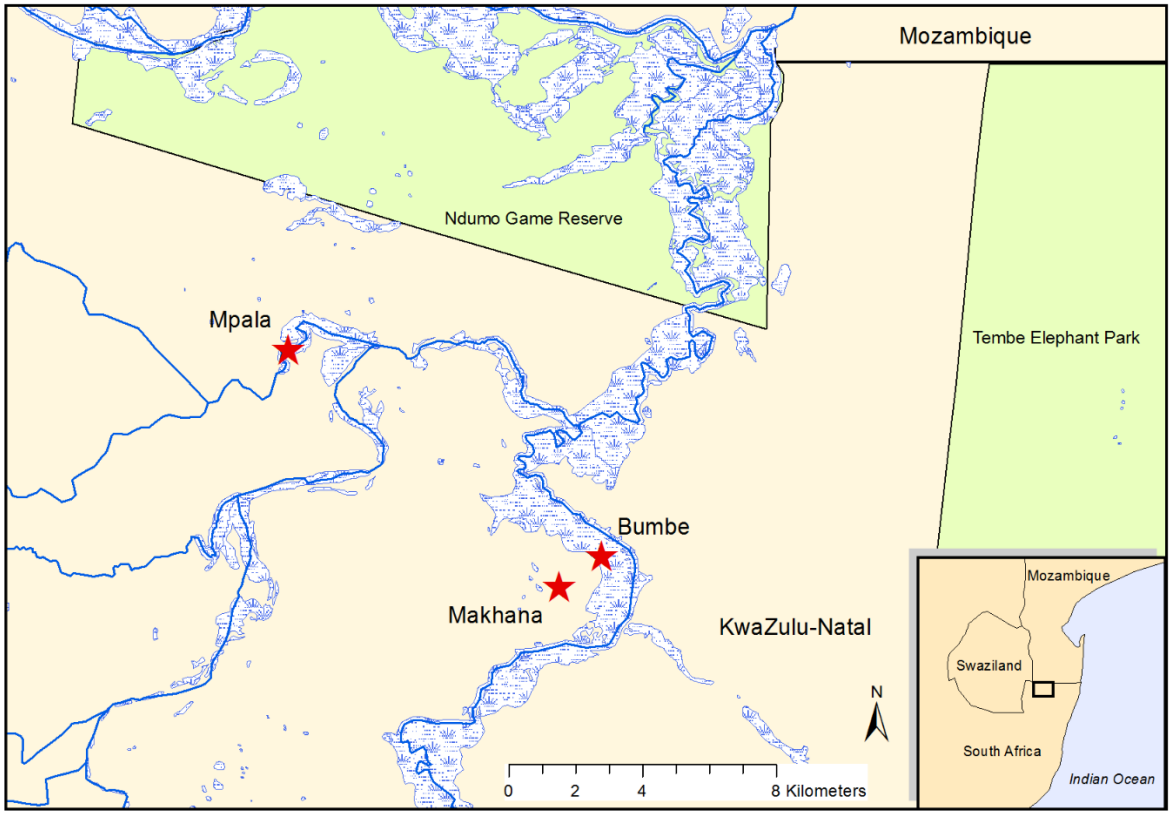


Figure 4.4: Map of Ndumo area showing the collection sites, rivers, maximum extent of the floodplains and the wildlife reserves.

4.2.3 Collection method

Adult mosquitoes were collected monthly, with 13 visits at 1-3 monthly intervals between November 2016 and June 2018 (6 visits for Makhana pan, from October 2017). Each visit consisted of 1-3 collections per site. Carbon dioxide in the form of dry ice was used as bait for a minimum of 12 hours. Traps (2 m x 2 m x 2 m net tents) made from a fine mesh were placed and baited in the late afternoon just before sunset and emptied just before sunrise with the aid of battery-powered hand-held vacuum collectors. All collections were placed on dry ice and then examined microscopically on ice bricks for species assignment. Morphological species identification was carried

out using keys (Knight *et al*., 1977, Gillies and Coetzee, 1987, Jupp, 1996). In cases where identification to species was not possible, mosquitoes were identified to group or genus level. Mosquitoes were pooled (maximum pool size = 50) by species, sex, site and month collected, and were kept at -80°C until viral screening. Blood-fed specimens were pooled separately.

4.2.4 Viral screening

Mosquito pools (n = 627) of species that had previously been implicated as potential vectors of RVFV were selected. Pools were homogenized using 2 ml homogenizing tubes (Anatech) in a Precellys 24 bead mill homogenizer (Anatech) at 6000 rpm for 1 min and centrifuged in an Eppendorf 5430 at 300m rpm for 3 minutes at room temperature. The samples were stored at -80°C until further processing. Nucleic acid was extracted using the magnetic bead technology of the MagMAX total Nucleic Acid Isolation Kit (Applied Biosystems). The kits were used according to the manufacturer's instructions.

Partial nucleic acid sequences of the L segment of the RVFV genome were amplified by using a one-step RT-qPCR (VetMax Plus OneStep RT-PCR Kit, Applied biosystems), according to the manufacturer's protocol. The primers RVFL-2912FWDGG (5′-TGAAAATTCCTGAGACACATGG-3′) and RVFL2981REVAC (5′-ACTTCCTTGCATCATCTGATG3′) were used to amplify the initial diagnostic fragment as described by Bird *et al*. (2007).

4.2.5 Statistical analysis

Mosquito density was calculated for each visit, overall and by site, as the arithmetic mean of the catch per trap-night, for each species and genus. Weather data were obtained from Ndumo Game Reserve, about 10 km from each of the sites, and cumulative rainfall, average maximum temperature and average minimum temperature were calculated for the 15- and 30-day periods prior to each visit.

Negative binomial models were used to assess associations between the actual mosquito numbers caught per trap-night and the site, cumulative rainfall and average minimum and maximum temperatures. For rainfall and temperature variables, linearity was assessed by using quartiles as predictors and examining for monotonic changes in estimated coefficients; based on this, cumulative rainfall and average minimum temperature were modelled as quartiles and average maximum temperature was modelled as a continuous variable. Models were performed for total mosquitoes caught, for each genus, and for particular species of interest. For rainfall and temperatures, various models were used with different possible combinations of 15-day and 30-day measurements and the best was selected using the Akaike information criterion (AIC), with lower values indicating better model fit. Statistical analyses were done using Stata 15 (StataCorp, College Station, TX, USA) and statistical significance was assessed at $P < 0.05$.

4.3 RESULTS

A total of 34,848 mosquitoes were collected over the period from November 2016 to June 2018, of which 73% were of the *Culex* genus. Another 19% of the mosquitoes were *Aedes*, 3% *Mansonia*, and 4% *Anopheles* and other genera. The identifications to species level are summarised in Table 4.1.

Table 4.1: Mosquito species and the total numbers of mosquitoes collected over the three areas in the study period.

Species	Mpala site	Bumbe site	Makhana site	Total	%
Anopheles (*Ano*.) *coustani*	112	81	57	250	0.7%
An. (*Ano*.) *ziemanni*	45	4	14	63	0.2%
An. (*Cel*.) *funestus* grp	17	42	18	77	0.2%
An. (*Cel*.) *gambiae*	52	165	52	269	0.8%
An. (*Cel*.) *maculipalpis*	8	15	0	23	0.1%
An. (*Cel*.) *pharoensis*	93	57	22	172	0.5%
An. (*Cel*.) *pretoriensis*	8	23	27	58	0.2%
An. (*Cel*.) *rufipes*	12	134	24	170	0.5%
An. (*Cel*.) *squamosus*	107	4	103	284	0.8%
An. (*Cel*.) *theileri*	1	5	3	9	<0.1%
Anopheles spp.	75	35	2	112	0.3%
Aedes (*Adm*.) *albocephalus*	4	12	2	18	0.1%
Ae. (*Adm.) argenteopunctatus* grp	0	5	0	5	<0.1%
Ae. (*Adm*.) *cumminsii*	82	30	0	112	0.3%
Ae. (*Adm*.) *dentatus/leesoni*	0	1	0	1	<0.1%
Ae. (*Adm*.) *durbanensis*	2283	1508	292	4083	11.7%
Ae. *(Adm.) fowleri*	112	19	15	146	0.4%
Ae. (*Adm*.) *hirsutus*	0	2	0	2	<0.1%
Ae. (*Adm*.) *leesoni* grp	2	17	0	19	0.1%
Ae. (*Adm*.) *ochraceus*	15	33	32	80	0.2%
Ae. (*Adm*.) *subdentatus*	0	5	0	5	<0.1%
Ae. (*Adm*.) *veeniae*	0	1	0	1	<0.1%
Ae. (*Muc*.) *sudanensis*	25	153	1	179	0.5%
Ae. (*Neo*.) *aurovenatus*	1	0	0	1	<0.1%
Ae. (*Neo*.) *circumluteolus*	450	516	139	1105	3.2%
Ae. (*Neo*.) *mcintoshi*	658	128	36	822	2.4%
Ae. (*Ste*.) *aegypti*	3	10	0	13	<0.1%
Ae. (*Ste*.) *metallicus*	1	24	0	25	0.1%
Aedes spp.	99	48	0	147	0.4%
Coquillettidia chrysosoma	7	0	1	8	<0.1%
Cq. *metallica*	1	0	0	1	<0.1%

Culex (*Cux*.) *annulioris*	15	5	2	22	0.1%
Cx. (*Cux*.) *antennatus*	4421	5071	488	9980	28.6%
Cx. (*Cux*.) *decens*	1	0	0	1	<0.1%
Cx. (*Cux*.) *ethiopicus*	1	1	2	4	<0.1%
Cx. (*Cux*.) *neavei*	886	1938	603	3427	9.8%
Cx. (*Cux*.) *perfuscus*	31	128	9	168	0.5%
Cx. (*Cux*.) *pipiens*	115	52	2	169	0.5%
Cx. (*Cux*.) *poicilipes*	240	27	52	319	0.9%
Cx. (*Cux*.) *simpsoni*	8	1	0	9	<0.1%
Cx. (*Cux*.) *sitiens*	1	0	0	1	<0.1%
Cx. (*Cux*.) *thalassius*	4	3	0	7	<0.1%
Cx. (*Cux*.) *theileri*	1	2	0	3	<0.1%
Cx. (*Cux*.) *tritaeniorhynchus*	5180	5404	375	10959	31.4%
Cx. (*Cux*.) *vansomereni*	0	2	0	2	<0.1%
Cx. (*Cux*.) *zombaensis*	40	138	13	191	0.5%
Cx. (*Lut*.) *tigripes*	50	0	0	5	<0.1%
Culex spp.	91	40	2	133	0.4%
Mansonia (*Man*.) *africana*	396	108	25	529	1.5%
Ma. (*Man*.) *uniformis*	473	156	27	656	1.9%
Mimomyia plumosa	0	0	1	1	<0.1%
Uranotaenia sp.	0	0	1	1	<0.1%

The collection per trap night numbers over the study period were plotted against the rainfall in the area over the study period (Fig. 4.5). As expected, the number of mosquitoes per trap night increased markedly after increased rainfall. During the winter months of June – August 2017 no mosquitoes were collected at each collection effort. The numbers only increased markedly again in March 2018 after high levels of rain fell during February 2018.

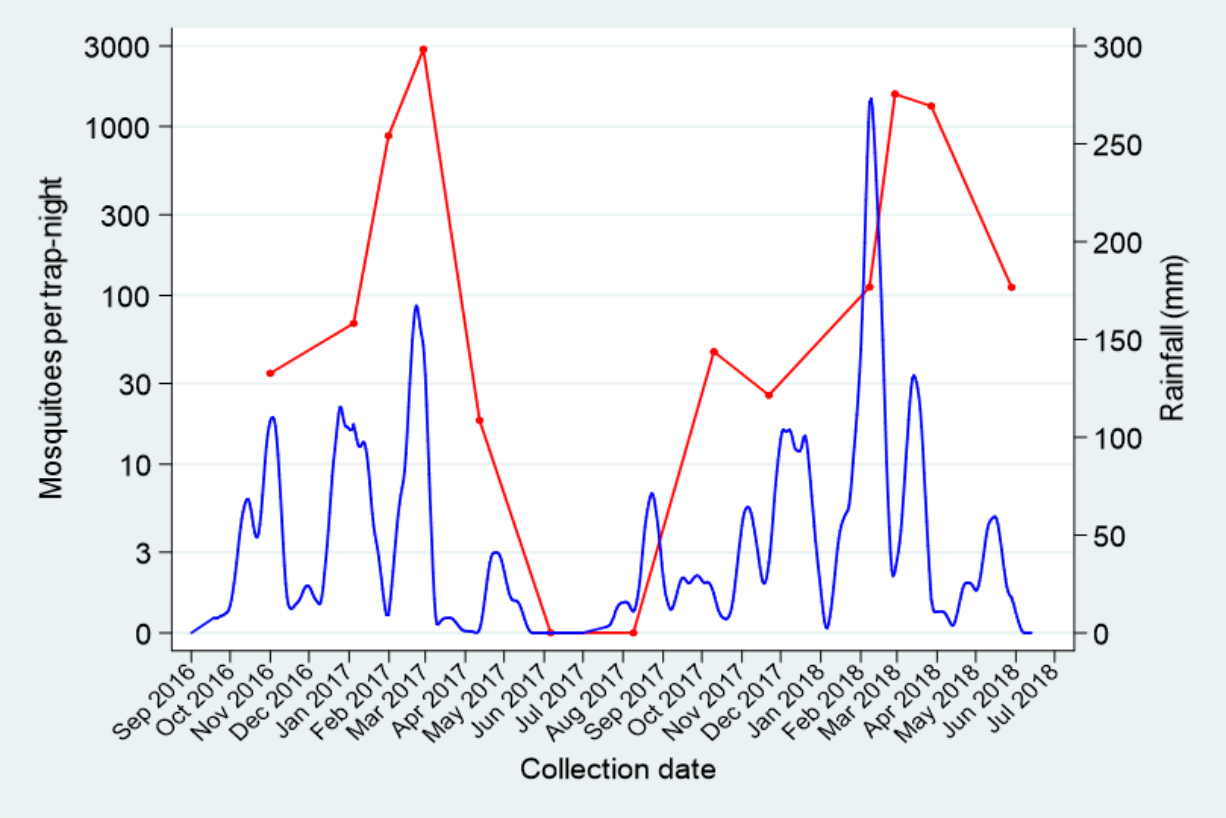


Figure 4.5: Graphical illustration of the total collections per trap night (red) as well as the rainfall (blue) over the entire collection period. The rainfall is indicated by a Lowess-smoothed curve of the cumulative rainfall over the preceding 15 days.

Peak densities differed among the sites; in March 2017 the peak density at the Mpala site (total count per trap night = 3331) was higher than the density at the Bumbe site (total count per trap night = 2415), while in 2018 the peak density at the Mpala site (total count per trap night = 805) was lower than the density at the Bumbe site (total count per trap night = 3247), but the peak density at the third location Makhana (total count per trap night = 624) was the lowest (Fig. 4.6).

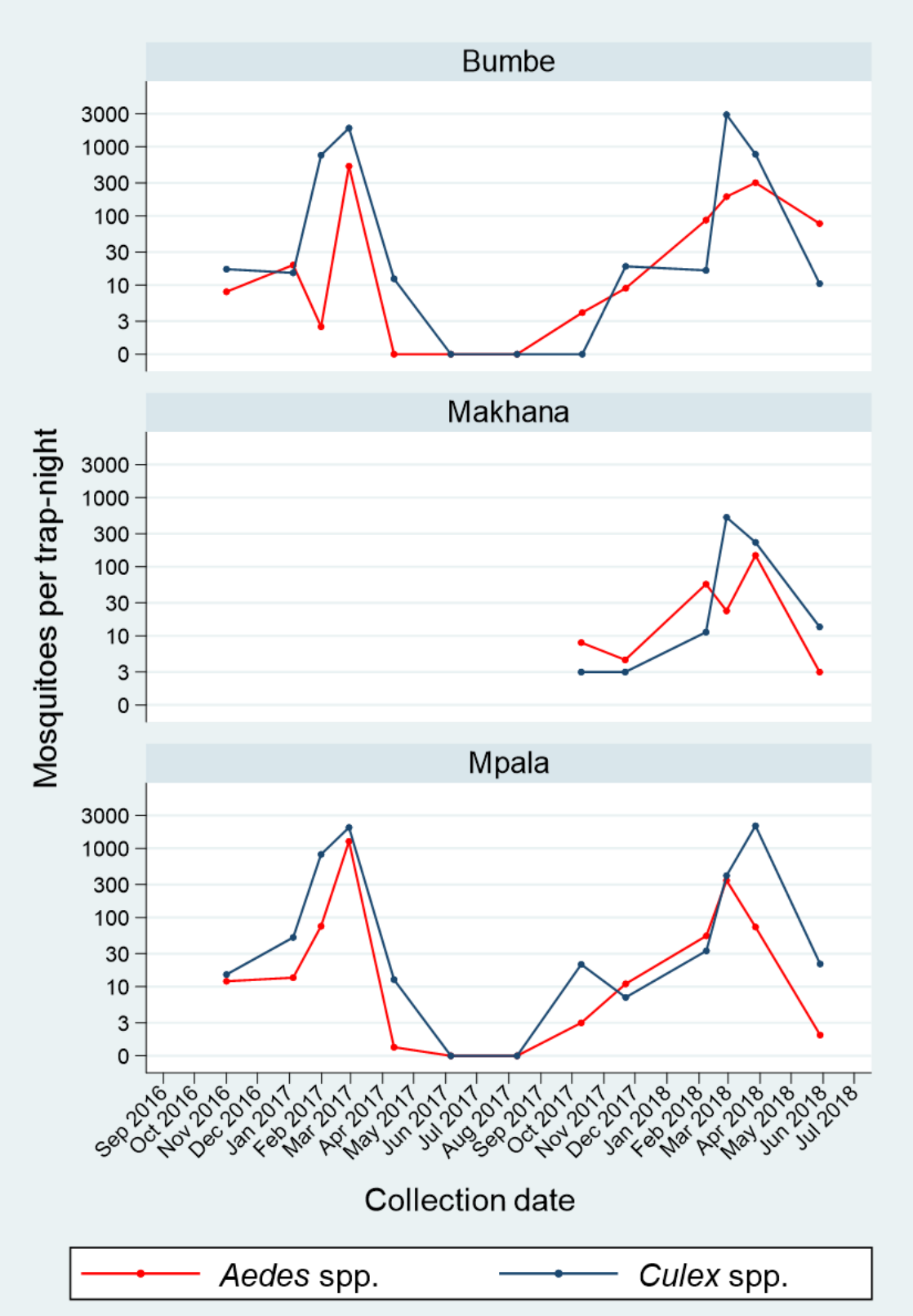
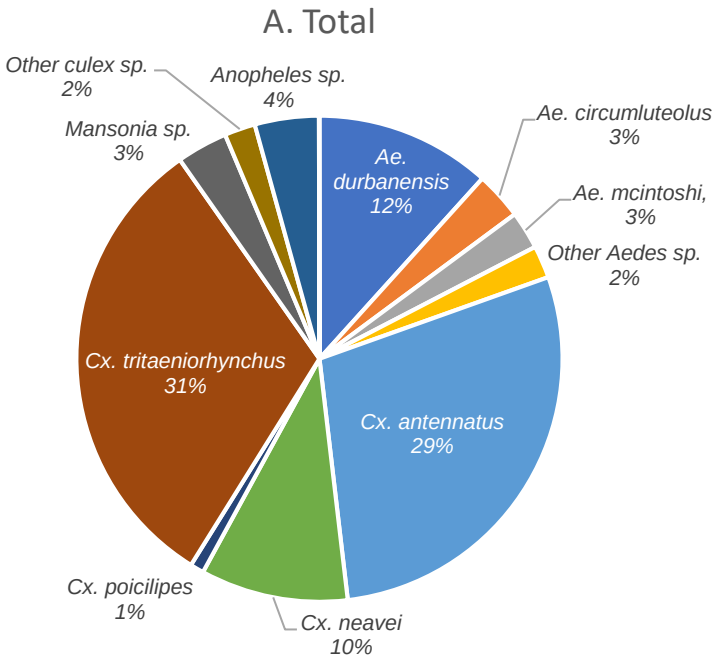


Figure 4.6: Mean number of *Aedes* and *Culex* mosquitoes collected per trap night for each of the three collection sites over the collection period.

Species distribution was not significantly different between the collection sites; *Cx. (Cux.) tritaeniorhynchus* was the most abundant species at all three collection sites, followed by *Cx. (Cux.) antennatus.* Makhana pan was the exception here, as *Cx. (Cux.) neavei* was the second most abundant species. *Aedes (Adm.) durbanensis* was the most abundant *Aedes* species at all three locations.

Genera distribution was different between the locations. *Aedes* spp. were more abundant at Mpala collection site than at Makhana and Bumbe collection sites; *Culex* spp. were most dominant at the Bumbe collection site while *Anopheles* spp. were proportionally most numerous at the Makhana collection site. The mosquito species percentages caught over the study period are illustrated in Fig. 4.7 A-D.



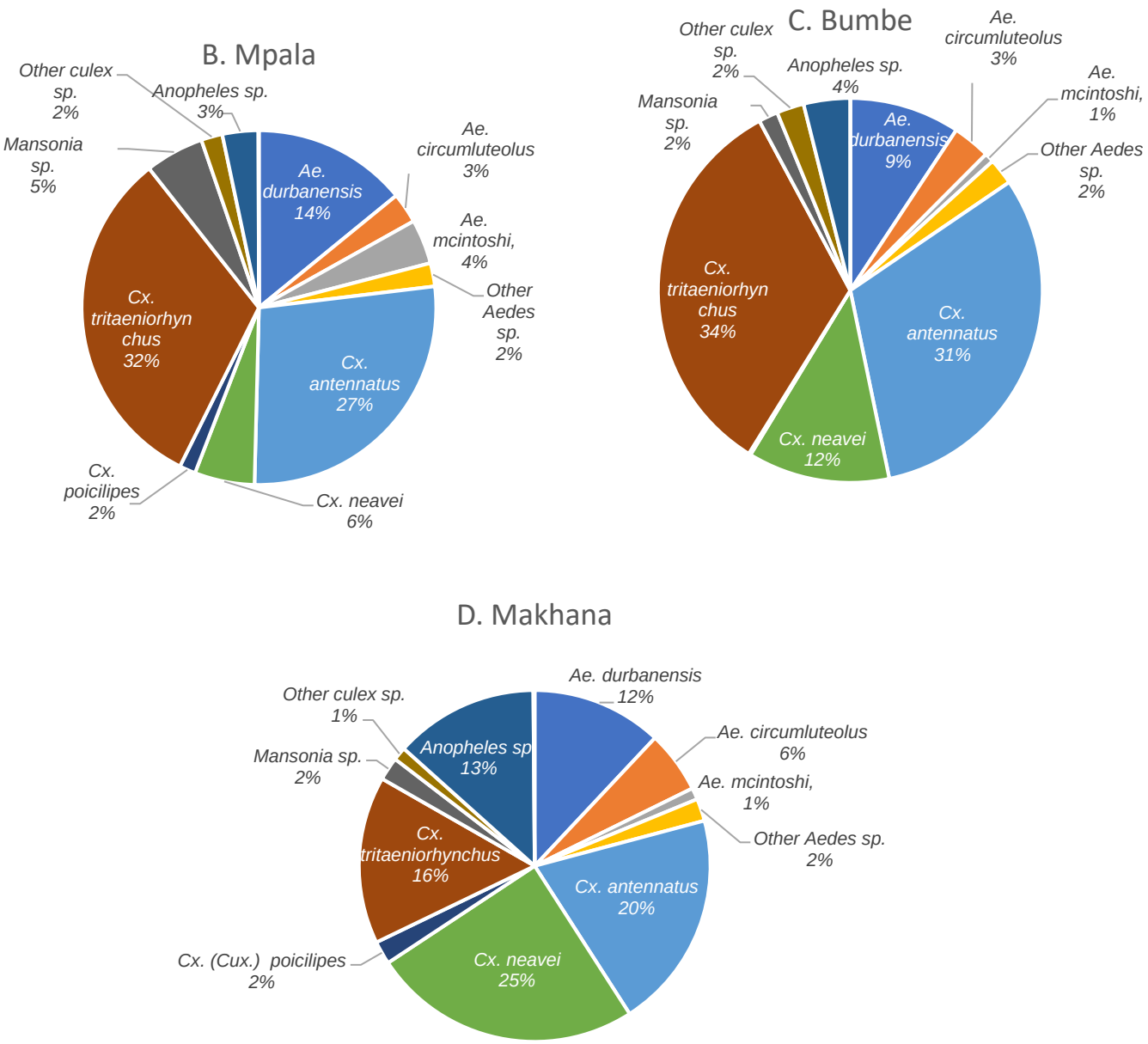


Figure 4.7: Relative mosquito species distribution: total (A), Mpala (B), Bumbe (C) and Makhana (D).

For all mosquito genera and groups, the multivariable models using 30-day cumulative rainfall and average temperature fitted better than those using 15-day values, based on AIC. The total rainfall 30 days prior to the collection was positively associated with

the total number of mosquitoes collected ($P < 0.001$), including *Aedes* ($P = 0.001$) and *Culex* ($P < 0.001$) (Table 4.2). For *Aedes* this increase plateaued after the third quartile (>100 mm) and for *Culex* it plateaued after the second quartile (20-100 mm). The average minimum temperature 30 days prior to collection had a significant positive association with the number *Aedes* spp. collected ($P < 0.001$), once it exceeded about 22°C, but was not noticeably associated with *Culex* numbers. The average maximum temperature 30 days prior to collection had a significant positive association with the total number of *Culex* mosquitoes collected ($P < 0.001$) but was not significantly associated with the number of *Aedes* or the total number of mosquitoes collected.

Table 4.2: Associations of total rainfall, and average minimum and maximum temperatures 30 days prior to collection of the *Aedes* and *Culex* genera and the total number of mosquitoes collected per trap-night at three sites in northern KZN, using negative binomial regression models. Significant ($P < 0.05$) count ratios are indicated in bold.

Variable	Count ratio (95% CI)		
	Aedes [a]	***Culex*** [b]	**Total collection** [c]
Site			
Mpala	1*	1*	1*
Bumbe	1.3 (0.6 - 2.9)	0.8 (0.5 - 1.5)	1.1 (0.6 - 2.0)
Makhana	**0.4 (0.2 - 1.1)**	**0.3 (0.2 - 0.7)**	**0.3 (0.1 - 0.7)**
Rainfall			
<20 mm	1*	1*	1*
20 - 100 mm	**1335 (106 - 16761)**	**150 (53.3 - 422)**	**451 (150 - 1354)**
100 - 230 mm	**4615 (426 - 49908)**	**138 (50.7 - 377)**	**298 (108 - 816)**
>230 mm	**2543 (234 - 27580)**	**76.6 (31.3 - 188.3)**	**127 (44.7 - 362)**
Minimum temperature			
15.7 - 17.8°C	1*	1*	1*
20 -21.7°C	2.0 (0.1 - 31.0)	**0.042 (0.009 - 0.2)**	0.5 (0.1 - 2.8)
21.9 - 23.6°C	**55.6 (2.1- 1457)**	4.7 (0.7 - 32.5)	**57.1 (7.0- 467)**
23.8 - 24°C	**18.5 (2.4 - 927)**	0.1 (0.01 - 1.5)	8.2 (0.6 - 122)
Maximum temperature			
	0.8 (0.5 - 1.2)	**1.7 (1.3 - 2.3)**	0.988 (0.7 - 1.3)

[a] AIC = 2399.0; [b] AIC = 3021.1; [c] AIC = 8380.6; *Reference category

From a total of 627 pools that were screened for RVFV there was only one pool (0.16%) that tested positive for RVFV. This was a pool of 50 *Ae. (Adm.) durbanensis* collected at Mpala during March 2017. The minimum infection rate (MIR) for *Ae. (Adm.) durbanensis* at Mpala for the March collection was 0.46 per 1000 mosquitoes and for *Ae. (Adm.) durbanensis* in the whole study area the MIR was 0.33 per 1000.

4.4 DISCUSSION

The interactions of arboviruses and their vectors are complex. The epidemiology of RVFV is poorly understood and relatively little is known about current *culicine* mosquito populations, the main vectors of RVFV, in northern KZN, an area of endemic RVFV circulation. This study was conducted to shed more light on the epidemiology of RVFV by investigating the potential vector species that occur in the area, as well as their abundance, and how they are impacted by season, temperature and rainfall.

To our knowledge this is the first study to report on mosquito population dynamics in a livestock area in KZN, South Africa. A previous study in 1967/8 reported on *Ae. (Neo.) circumluteolus* in an area mainly consisting of wildlife in Ndumo Game Reserve (Jupp and McIntosh, 1987), but no recent studies have been done in the far northern part of KZN.

This study was done in an area in South Africa where no outbreaks of RVF have been reported. A longitudinal study in the same area, during the same study period showed high levels of exposure to RVFV (Chapter 2). The seroprevalence in cattle in June 2016 was 34% and in goats in February 2017 it was 31.7% (Van den Bergh, *et al*., 2019). The study area is a low lying, tropical region from which *Bunyamwera* orthobunyavirus and RVFV (Kokernot *et al*., 1957a), Spondweni virus (Kokernot *et al*., 1957b), Pongola virus (Kokernot *et al*., 1957a), Ndumu virus (Kokernot *et al*., 1961) and Wesselsbron virus (Muspratt *et al*., 1957), amongst others, have been isolated in previous studies. The human and livestock population in the area has increased significantly over time, resulting in an increased RVFV host density and possible changes in the vector population composition and dynamics.

A total of 34,848 mosquitoes were collected over the period from November 2016 to June 2018, with the most prevalent species being *Cx. (Cux.) tritaeniorhynchus* followed by *Cx. (Cux.) antennatus.* This is consistent with the habitat since permanent water sources were present at all collection sites. The most prevalent *Aedes* species were *Ae. (Adm.) durbanensis* followed by *Ae. (Neo.) circumluteolus* and *Ae. (Neo.) mcintoshi*. During previous studies both *Ae. (Adm.) durbanensis* and *Ae. (Neo.) circumluteolus* were caught around the northern coastal shores of Natal (KZN), and in Mozambique the most predominant species was *Ae. (Neo.) circumluteolus* (McIntosh, 1972). However, in our study the most predominant species were *Cx. (Cux.) tritaeniorhynchus* (31%), followed by *Cx. (Cux.) antennatus* (28%), *Ae. (Adm.) durbanensis* (12%), and *Cx. (Cux.) neavei* (10%), with only 3% of *Ae. (Neo.) circumluteolus.* This may be due to the use of different bait techniques, since live bait including sheep and humans were used in the previous study. Also, the present study different areas were sampled. The collection sites were not in the nature reserves as with the previous study but among livestock and closer to human settlements. The difference in collection numbers might also be due to bloodmeal preferences, since no wildlife were present near any of the collection sites, as they were in the previous study (McIntosh, 1972). In addition, the long period of time (40-50 years) elapsed since the previous studies and the marked increase in the human and livestock population over that period, makes it likely that the mosquito population composition in the area has also changed. Further work is required to investigate the current difference in mosquito populations between wildlife and livestock areas in order to determine the extent to which the increase in livestock population has impacted mosquito population composition and diversity.

The relative species distribution at the three study sites was different, which might be because of the differences in habitat between the different collection sites. *Culex (Cux.) antennatus* was the second most abundant species at the Mpala and Bumbe collection sites while the second most abundant mosquito at Makhana collection site was *Cx. (Cux.) neavei.* This might be due to bloodmeal preference since *Cx. (Cux.) antennatus* prefers to feed on livestock (Gad *et al*., 1995), in a previous study, *Cx. (Cux.) neavei* seemed to prefer feeding on humans, horses and chickens (McIntosch *et al*., 1972, Diallo *et al*., 2010). The Makhana collection site was in a human

settlement with free-roaming chickens which might explain the elevated numbers of *Cx. (Cux.) neavei.*

Floodwater *Aedes* spp. which include the *Aedimorphus, Mucidus, Neomelaniconion,* and *Ochlerotatus* subgenera, lay their eggs in grassland when it becomes inundated after heavy rainfall, such as at the edges of pans, flood plains, dams and rivers. Before the next rainy season or episode of flooding the eggs become dry, which is a necessary step for them to mature and be able to hatch. The eggs can survive for long periods of time, possibly even years (Gargan II *et al*., 1988). When the breeding habitat re-floods in a subsequent rainy season the eggs hatch and the mosquitoes mature (Jupp, 1996). *Culex* spp., on the other hand, oviposit their eggs in permanent and to a lesser extent temporary standing water, and the eggs are not resistant to desiccation (Jupp, 1996, Jupp and McIntosh, 1967). The Bumbe collection site had trees around the nets and the immediate pan had no water during the collection period. However, the Pongola river, about 500 m away from the collection site was a permanent source of water. *Culex* was the dominant genus collected at this site. The numbers of *Culex* mosquitoes were lower for Mpala where a small inlet to the Qotho pan served as the only permanent water source and was at times almost dry. *Aedes* collection totals were higher at Mpala, where, although the Qotho pan was largely dry, it provided a large, flat area where rainfall created numerous small, temporary areas of standing water. This likely gave rise to more frequent hatching of *Aedes* eggs compared to Bumbe.

Mansonia species were more abundant at the Mpala location, which is consistent with the vegetation around the Qotho pan and the inlet of the Ingwavuma river, with numerous water plants including water lilies (*Nymphaea* spp.). This creates a suitable breeding habitat for *Mansonia* mosquitoes since females lay their eggs underneath the leaves of water plants such as these and *Pistia striatotes* (Jupp, 1996).

For all mosquito groups, the numbers caught were better correlated with rainfall and temperature over the 30 days prior to collection than with conditions over only 15 days prior to collection. This is naturally explained by the fact that ideal conditions for proliferation of mosquitoes, including maturation of breeding sites and growth of surrounding vegetation, is likely to take longer to develop than just the 15 days

required for larval development. Moreover, more than one generation may be needed to build up the population. In satellite-derived estimation of environmental suitability for malaria vector development, model efficiency was higher when based on static variables (distances to breeding sites and land cover), as well as on dynamic variables such as enhanced vegetation index and temperature, over a 2 to 4 week interval (Benali *et al*., 2014). This suggests that multiple factors in the ecosystem, including temperature, rainfall and vegetation productivity over a period of longer than 2 weeks are important determinants of mosquito population dynamics. The total number of *Aedes* spp. collected was affected by the average minimum temperature 30 days before the collection period. This was different for *Culex* spp. which were affected more by the average maximum temperature 30 days before the collection period. The reason for these associations is not fully understood and more research is required to understand which factors influence the mosquito population abundance.

Rift Valley fever virus has been isolated from at least 40 different mosquito species (Meegan and Bailey, 1989, Fontenille *et al*., 1998, Pepin *et al*., 2010, Seufi and Galal, 2010). Vector competence has been shown in laboratory environments in several species including *Ae. (Neo.) mcintoshi*, *Ae. (Ste.) aegypti*, *Cx. (Cux.) antennatus*, *Cx. (Cux.) pipiens, Cx. (Cux.) poicilipes* (Turell *et al*., 2008), *Ae. (Adm.) ochraceus* (Gear *et al*., 1955) (McIntosh, 1972) and *Cx. (Cux.) zombaensis* (McIntosh *et al*., 1983), most of which were collected at all sites during the study period. In addition, several other species implicated as RVFV vectors elsewhere were found in large numbers during the present study, including *Cx. (Cux.) tritaeniorhynchus*, a known RVFV vector in Saudi Arabia and Yemen in 2000 (Miller *et al*., 2002); *Cx. (Cux.) antennatus*, a vector in Egypt (Gad *et al*., 1995); *Cx. (Cux.) neavei*, a vector in South Africa in 1981 (McIntosh and Jupp, 1981) and in Mauritania in 2003 (Faye *et al*., 2007); *Ae.(Neo.) circumluteolus*, from which RVFV was isolated in this area in 1955 (Kokernot *et al*., 1957a); *Ae. (Adm.) durbanensis,* implicated in causing RVF disease symptoms in sheep in Kenya in 1937 (Steyn and Schulz, 1955); and *Ae. (Neo.) mcintoshi*, in which transovarial transmission was demonstrated in Kenya (Linthicum *et al*., 1985).

Results obtained in this study suggest that several mosquito species that could potentially be vectors for RVFV appear in the study area, firstly due to their incrimination as known or suspected vectors elsewhere, and secondly due to their occurrence in large numbers. These include *Cx. (Cux.) tritaeniorhynchus*, *Cx. (Cux.) antennatus, Cx. (Cux.) neavei, Ae. (Neo.) circumluteolus* and *Ae. (Adm.) durbanensis*, in which RVFV was detected during the present study. The detection of RVFV occurred during the peak time of RVFV transmission in cattle (Chapter 2), which suggests that this mosquito may have played a role in virus transmission amongst livestock in the area, since the mosquitoes that were screened were all unfed females, ruling out potential contamination with infected blood. The mosquitoes were also collected at Mpala collection site, an ideal breeding environment for floodwater breeders since the water levels fluctuated over the study period. Further investigation is required to determine whether *Ae. (Adm.) durbanensis* is capable of transovarial transmission.

REFERENCES

1. ADAMS, M. J., LEFKOWITZ, E. J., KING, A. M., HARRACH, B., HARRISON, R. L., KNOWLES, N. J., KROPINSKI, A. M., KRUPOVIC, M., KUHN, J. H. & MUSHEGIAN, A. R. 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Archives of Virology,* 162**,** 2505-2538.
2. BENALI, A., NUNES, J., FREITAS, F., SOUSA, C., NOVO, M., LOURENÇO, P., LIMA, J., SEIXAS, J. & ALMEIDA, A. 2014. Satellite-derived estimation of environmental suitability for malaria vector development in Portugal. *Remote Sensing of Environment,* 145**,** 116-130.
3. BIRD, B. H., BAWIEC, D. A., KSIAZEK, T. G., SHOEMAKER, T. R. & NICHOL, S. T. 2007. Highly sensitive and broadly reactive quantitative reverse transcription-PCR assay for high-throughput detection of Rift Valley fever virus. *Journal of Clinical Microbiology,* 45**,** 3506-3513.
4. DAVIES, F., LINTHICUM, K. & JAMES, A. 1985. Rainfall and epizootic Rift Valley fever. *Bulletin of the World Health Organization,* 63**,** 941.
5. DIALLO, D., BA, Y., DIA, I., SALL, A. & DIALLO, M. 2010. Evaluation of the efficiency of bird-baited traps for sampling potential West Nile fever mosquito vectors (Diptera: Culicidae) in Senegal. *Parasites,* 17**,** 143-147.
6. EVANS, A., GAKUYA, F., PAWESKA, J., ROSTAL, M., AKOOLO, L., VAN VUREN, P. J., MANYIBE, T., MACHARIA, J., KSIAZEK, T. & FEIKIN, D. 2008. Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife. *Epidemiology and Infection,* 136**,** 1261-1269.
7. FAFETINE, J. M., COETZEE, P., MUBEMBA, B., NHAMBIRRE, O., NEVES, L., COETZER, J. A. & VENTER, E. H. 2016. Rift Valley fever outbreak in livestock, Mozambique, 2014. *Emerging Infectious Diseases,* 22**,** 2165.
8. FAYE, O., DIALLO, M., DIOP, D., BEZEID, O. E., BÂ, H., NIANG, M., DIA, I., MOHAMED, S. A. O., NDIAYE, K. & DIALLO, D. 2007. Rift Valley fever outbreak with East-Central African virus lineage in Mauritania, 2003. *Emerging Infectious Diseases,* 13**,** 1016.
9. FONTENILLE, D., TRAORE-LAMIZANA, M., DIALLO, M., THONNON, J., DIGOUTTE, J. & ZELLER, H. 1998. New vectors of Rift Valley fever in west Africa. *Emerging Infectious Diseases,* 4**,** 289.
10. GAD, A. M., RIAD, I. B. & FARID, H. A. 1995. Host-feeding patterns of Culex pipiens and Cx. antennatus (Diptera: Culicidae) from a village in Sharqiya Governorate, Egypt. *Journal of Medical Entomology,* 32**,** 573-577.
11. GARGAN II, T. P., CLARK, G. G., DOHM, D. J., TURELL, M. J. & BAILEY, C. L. 1988. Vector potential of selected North American mosquito species for Rift Valley fever virus. *The American Journal of Tropical Medicine and Hygiene,* 38**,** 440-446.
12. GEAR, J., DE MEILLO, B., LE ROUX, A., KOFSKY, R., ROSE, R., STEYN, J., OLIFF, W. & SCHULZ, K. 1955. Rift valley fever in South Africa: A study of the 1953 outbreak in the Orange Free State, with special reference: To the vectors and possible reservoir hosts. *South African Medical Journal,* 29.
13. GILLIES, M. & COETZEE, M. 1987. A supplement to the Anophelinae of Africa South of the Sahara. *Publ South African Institute of Medical Research,* 55**,** 1-143.
14. JUPP, P. & CORNEL, A. 1988. Vector competence tests with Rift Valley fever virus and five South African species of mosquito. *Journal of the American Mosquito Control Association,* 4**,** 4-8.
15. JUPP, P. & MCINTOSH, B. 1967. Ecological studies on Sindbis and West Nile viruses in South Africa. II. Mosquito bionomics. *The South African Journal of Medical Sciences,* 32**,** 15.

16. JUPP, P. & MCINTOSH, B. 1987. A bionomic study of adult Aedes (Neomelaniconion) circumluteolus in northern Kwazulu, South Africa. *Journal of the American Mosquito Control Association,* 3**,** 131-136.
17. JUPP, P. G. 1996. *Mosquitoes of Southern Africa: culicinae and toxorhynchitinae*, Ekogilde Publishers.
18. KNIGHT, K. L., STONE, A. & STONE, A. 1977. catalog of the mosquitoes of the world (Diptera, Culicidae).
19. KOKERNOT, R., HEYMANN, C., MUSPRATT, J. & WOLSTENHOLME, B. 1957a. Studies on arthropod-borne viruses of Tongaland. V. Isolation of Bunyamwera and Rift Valley Fever viruses from mosquitoes. *South African Journal of Medical Sciences,* 22**,** 71-80.
20. KOKERNOT, R., MCINTOSH, B. & WORTH, C. B. 1961. Ndumu virus, a hitherto unknown agent, isolated from culicine mosquitoes collected in northern Natal, Union of South Africa. *The American Journal of Tropical Medicine and Hygiene,* 10**,** 383-386.
21. KOKERNOT, R., SMITHBURN, K., MUSPRATT, J. & HODGSON, B. 1957b. Studies on arthropod-borne viruses of Tongaland. VIII. Spondweni virus, an agent previously unknown, isolated from Taeniorhynchus (Mansonioides) uniformis Theo. *South African Journal of Medical Sciences,* 22**,** 103-112.
22. LINTHICUM, K., DAVIES, F., KAIRO, A. & BAILEY, C. 1985. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Epidemiology and Infection,* 95**,** 197-209.
23. MCINTOSCH, J., JUPP, P. & SOUSA, D. 1972. Mosquitoes feeding at two horizontal levels in gallery forest in Natal. South Africa. with reference to possible vectors of chikungunya virus. *Journal of the Entomological Society of Southern Africa,* 35**,** 81-90.
24. MCINTOSH, A., JUPP, P. & DOS SANTOS, I. 1983. Field and laboratory evidence implicating Culex zombaensis and Aedes circumluteolus as vectors of Rift Valley fever virus in coastal South Africa. *South African Journal of Science,* 79**,** 61-64.
25. MCINTOSH, B. 1972. Rift valley fever: 1. Vector studies in the field. *Journal of the South African Veterinary Association,* 43**,** 391-395.
26. MCINTOSH, B. & JUPP, P. Epidemiological aspects of Rift Valley fever in South Africa with reference to vectors. Proceedings... Rift Valley fever; a workshop, 1981.
27. MCINTOSH, B., JUPP, P., DOS SANTOS, I. & BARNARD, B. 1980. Vector studies on Rift Valley fever virus in South Africa. *South African Medical Journal,* 58**,** 127-32.
28. MCLNTOSH, B., JUPP, P. & DE SOUSA, J. 1972. Further isolations of arboviruses from mosquitoes collected in Tongaland, South Africa, 1960–1968. *Journal of Medical Entomology,* 9**,** 155-159.
29. MEEGAN, J. & BAILEY, C. 1989. Rift Valley Fever. 51-76. *In:* MONATH, T.P., (ed.). *The Arboviruses: Epidemiology and Ecology.* Vol. lV. Boca Raton, Florida:CRC Press Inc. pp. 51-57.
30. MEEGAN, J. M. & SHOPE, R. E. 1981. Emerging concepts on Rift Valley fever. *Perspect. Virology,* 11**,** 267-387.
31. MILLER, B. R., GODSEY, M. S., CRABTREE, M. B., SAVAGE, H. M., AL-MAZRAO, Y., AL-JEFFRI, M. H., ABDOON, A.-M. M., AL-SEGHAYER, S. M., AL-SHAHRANI, A. M. & KSIAZEK, T. G. 2002. Isolation and genetic characterization of Rift Valley fever virus from Aedes vexans arabiensis, Kingdom of Saudi Arabia. *Emerging Infectious Diseases,* 8**,** 1492.
32. MOIANE, B., MAPACO, L., THOMPSON, P., BERG, M., ALBIHN, A. & FAFETINE, J. 2017. High seroprevalence of Rift Valley fever phlebovirus in domestic ruminants and African buffaloes in Mozambique shows need for intensified surveillance. *Infection Ecology and Epidemiology,* 7**,** 1416248.
33. MOLL, E. 1980. Terrestrial plant ecology. *Studies on the Ecology of Maputaland***,** 52-68.

34. MUSPRATT, J., SMITHBURN, K., PATERSON, H. & KOKERNOT, E. 1957. Studies on arthropod-borne viruses of Tongaland. X. The laboratory transmission of Wesselsbron virus by the bite of Aedes (Banksinella) circumluteolus Theo. *South African Journal of Medical Sciences,* 22**,** 121-126.
35. PEPIN, M., BOULOY, M., BIRD, B. H., KEMP, A. & PAWESKA, J. 2010. Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Veterinary Research,* 41**,** 61.
36. ROGERS, K. 1980. The vegetation of the Pongolo floodplain: distribution and utilization. *Studies of the Ecology of Maputaland***,** 69-77.
37. ROLIN, A. I., BERRANG-FORD, L. & KULKARNI, M. A. 2013. The risk of Rift Valley fever virus introduction and establishment in the United States and European Union. *Emerging Microbes & Infections,* 2**,** 1-8.
38. ROSSOUW, J. 1985. The effects of the Domoina floods and releases from the Pongolapoort Dam on the Pongolo floodplain. *Unpublished report. File no. B N,* 3. (http://www.dwa.gov.za/IWQS/reports/tr/B-N3_0704_Domoina%20floods%20and%20the%20Pongolo%20floodplain.pdf)
39. SEUFI, A. M. & GALAL, F. H. 2010. Role of Culex and Anopheles mosquito species as potential vectors of rift valley fever virus in Sudan outbreak, 2007. *BMC Infectious Diseases,* 10**,** 65.
40. STEYN, J. & SCHULZ, K. 1955. Aëdes (Ochlerotatus) caballus Theobald, the South African vector of Rift Valley fever. *South African Medical Journal,* 29**,** 1114-20.
41. TURELL, M. J., LINTHICUM, K. J., PATRICAN, L. A., DAVIES, F. G., KAIRO, A. & BAILEY, C. L. 2008. Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley fever virus. *Journal of Medical Entomology,* 45**,** 102-108.
42. VAN DEN BERGH, C., VENTER, E. H., SWANEPOEL, R. & THOMPSON, P. N. 2019. High seroconversion rate to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal, South Africa, in the absence of reported outbreaks. *PLOS Neglected Tropical Diseases,* 13**,** e0007296.

Chapter 5

Detection of Rift Valley fever, Chikungunya and Sindbis viruses in *Aedes (Aedimorphus) durbanensis* from northern KwaZulu-Natal, South Africa

This chapter is written in preparation for publication – (therefore the repetition of general facts).

5.1 INTRODUCTION

African arboviruses belong to families *Flaviviridae, Togaviridae, Reoviridae* and *Phenuiviridae,* and many of them are associated with fever and abortions in animals and with mild febrile illness that may progress to haemorrhagic fever, encephalitis, and death in humans (Venter, 2018, Weaver and Reisen, 2010, Hollidge *et al*., 2010). These viruses survive using a complex cycle of vertebrate hosts, amplifying or maintenance hosts or reservoir and competent arthropod vectors, which may include a wide variety of arthropods such as mosquitoes, biting midges, sand flies, and ticks as maintenance hosts (Mackenzie and Jeggo, 2013). They replicate in the arthropod vector to produce high viral loads in the salivary glands and are then transmitted to vertebrate hosts during feeding (Pfeffer and Dobler, 2010). Many arboviruses circulate among wildlife with spill over to humans and domestic animals, which may act as incidental or dead-end hosts. Many arboviruses can cause outbreaks resulting in significant health and socio-economic losses (Homenauth *et al*., 2017).

Rift Valley fever virus (RVFV) (family *Phenuiviridae*, genus *Phlebovirus*) has caused severe intermittent and sporadic outbreaks in domestic ruminants in sub-Saharan Africa (Adams *et al*., 2017). Rift Valley fever (RVF) is a disease usually recognized by the onset of abortion storms and high mortality rates in young ruminants (Morrill *et al*., 1997). The first outbreak in South Africa was recognized in 1951 in the western Free State, South and South western Transvaal (North West Province) and the North-Western Cape during the autumn season (Gear *et al*., 1951). Transmission studies proved that *Culex* (*Cux.*) *theileri, Cx.* (*Cux.*) *zombaensis, Cx.* (*Cux.*) *neavei* and

Eretmapodites quinquevittatus were able to transmit the disease successfully, while *Anopheles* (*Ano.*) *coustani*, *Ae.* (*Neo.*) *mcintoshi* and *Ae.* (*Neo.*) *circumluteolus* failed to transmit the virus (McIntosh *et al*., 1980). Transovarial transmission was suggested when RVFV was isolated in *Ae. mcintoshi* adults raised from larvae from an artificially flooded dambo in Kenya (Linthicum *et al*., 1985). In a later study *Ae. mcintoshi* and *Ae. circumluteolus* demonstrated competency as RVFV vectors when adult mosquitoes, infected at the larval stage were able to transmit the virus (Turell *et al*., 1990).

During an outbreak in Egypt (1977-1978) RVFV was isolated from indoor-resting *Culex* (*Cux.*) *pipiens (*Hoogstraal *et al., 1979).* The transmitting capability of the virus was then tested under laboratory conditions and it was confirmed that this species in the Nile Delta and Valley can be a vector for RVFV (Meegan *et al*., 1980). New vectors in West and Central Africa were identified and included *Ae.* (*Adm.*) *dalzieli, Ae. (Adm.*) *ochraceus, Ae.* (*Adm.*) *vexans*, *Ae.* (*Adm.*) *cumminsii, Ae. (Dic) furcifer, Ae. (Neo) palpalis, Cx. (Cux.) antennatus* and *Mansonia africana* (Fontenille *et al*., 1998). Rift Valley fever virus was isolated from *Cx.* (*Cux.*) *poicilipes* in 2000 for the first time in Mauritania (Diallo *et al*., 2000).

Two other major outbreaks have occurred in South Africa, in 1974-1976 in the northern Cape Province, Southern Orange Free State, western parts of the Transvaal (North West Province) and the Eshowe area in KwaZulu-Natal (KZN), and again in 2008-2011 affecting almost all provinces (Pienaar and Thompson, 2013).

The maintenance of the virus during long inter-epidemic periods, and whether vertebrate reservoirs are involved, is still uncertain. Transovarial transmission of the virus in floodwater-breeding *Aedes* spp. mosquitoes is currently regarded as the primary maintenance mechanism (Linthicum *et al*., 1985), along with low level circulation between vectors and hosts during IEPs. It has also been suggested that the virus can be reintroduced with the movement of infected hosts (Gad *et al*., 1986, Sellers *et al*., 1982)

Chikungunya virus (CHIKV) (Family *Togaviridae*, genus *Alphavirus*) has a single-stranded, positive sense RNA (Tsetsarkin *et al*., 2011). The virus was first isolated in Tanzania in 1952-53 during an outbreak among humans, who presented with febrile symptoms accompanied with arthralgia, rash and fever (Jupp, 1988). The disease caused by CHIKV is highly debilitating but not life-threatening and may often be misdiagnosed as dengue when proper laboratory diagnostics are not available, leading to under reporting (Carey, 1971, Charrel *et al*., 2007).

Outbreaks of Chikungunya have increased dramatically over the years. An outbreak occurred on the coast of Kenya in 2004 due to a virus strain belonging to the eastern, central, and southern African (ECSA) lineage (Schuffenecker *et al*., 2006, Chretien *et al*., 2007). The disease spread to the nearby islands of Comoros in 2005 and continued to other islands in the Indian Ocean. La Reunion was the worst affected with over 300 000 human patients infected. Later the following year the virus spread back to East Africa and India (Robin *et al*., 2008, Mavalankar *et al*., 2008). Chikungunya virus was introduced into Italy in 2007 by a traveller from India, and in 2010 transmission was recorded in south eastern France (Grandadam *et al*., 2011). It is suspected that the virus can spread into northern latitudes where *Aedes (Stegomyia) albopictus*, a competent vector, has become established (Weaver and Reisen, 2010). A virus belonging to an Asian lineage was introduced to the island of St. Martin in 2013 (Leparc-Goffart *et al*., 2014) and the virus has now spread through the Caribbean and to Central America, South America and Florida (Cauchemez *et al*., 2014).

Chikungunya virus has been recorded in South Africa in the eastern Transvaal lowveld (Mpumalanga Province) infecting humans as early as 1956, and again in 1975, 1976 and 1977 (Gear and Reid, 1957, McIntosh and Jupp, 1977, Morrison, 1979). An outbreak in vervet monkeys (*Chlorocebus pygerythrus*) was detected serologically in Ndumo Game Reserve, northern Natal (KZN) in 1964 (McIntosh, 1970). Further studies in the north-eastern Transvaal in 1976 have implicated the vervet monkey along with chacma baboons (*Papio ursinus*) as primary hosts of CHIKV and identified *Ae.* (*Dic.*) *furcifer* and *Ae.* (*Dic.*) *cordellieri* as the primary vectors in South Africa (McIntosh and Jupp, 1977). *Ae.* (*Ste.*) *aegypti*, *Ae.* (*Fin fulgens* and *Ae*. (*Fre.) vittatus* were shown to be competent laboratory vectors of CHIKV (McIntosh, 1970, Jupp and

McIntosh, 1990). The virus can be divided into three major lineages, including West Africa (WA), eastern, central and southern African (ECSA) and Asia. Additionally, the Indian Ocean lineage emerged within the eastern, central and southern African and an Asian/American lineage within the Asian lineage (Langsjoen *et al*., 2018).

Sindbis virus (SINV) (Family *Togaviridae*, genus *Alphavirus*) has a single-stranded, positive sense RNA genome (Strauss *et al*., 1984) with the most extensive geographical distribution of all alphaviruses. The virus activity has been reported in Africa including South Africa, Uganda, Cameroon and Egypt, Eurasia including Sweden, Finland, United Kingdom, India, Italy, Israel, Saudi Arabia, China and the Russian Federation, and Oceania including Malaysia, Australia and the Philippines (Lundström and Pfeffer, 2010). The virus circulates in transmission cycles between wild birds and mosquito vectors and is often accidentally transmitted to humans and other vertebrates. Migratory birds also transport the virus over long distances (Brummer-Korvenkontio *et al*., 2002, Lundström and Pfeffer, 2010).

Sindbis virus causes infection in humans during the summer months in South Africa and is widespread in areas such as Gauteng, Northern Cape and Free State with a few sporadic cases in KZN, Western Cape, Mpumalanga and the North West provinces (Storm *et al*., 2013, McIntosh *et al*., 1964, Uejio *et al*., 2012). The largest outbreak in South Africa was reported in 1974 in the Karoo and Northern Cape Province (McIntosh *et al*., 1976). Another outbreak was reported during 1983 and 1984 in Witwatersrand and Pretoria areas (Gauteng Province). Both the outbreaks involved *Cx. (Cux.) univittatus*, suggesting that it is the primary vector in the temperate inland plateau areas while *Cx. (Cux.) neavei,* another bird feeding species, serves as the vector in the coastal northern Natal (KZN) (McIntosh *et al*., 1976). The disease presents mostly as a febrile disease with symptoms such as arthritis, rash, fever, and nausea; no fatalities have been reported. Prolonged arthritis and musculoskeletal symptoms have been reported in patients during this outbreak (Kurkela *et al*., 2005).

The Sindbis complex is made up of multiple subtypes that include Ockelbo virus, Babanki virus, Karelian fever virus, Whataroa virus and Kyzylagach virus (Weaver *et*

al., 1997). Recently SINV has been subdivided in two lineages, namely Aura and Sindbis virus (Lundström and Pfeffer, 2010).

The Maputaland coastal plain in northern KZN has a tropical climate characterized by warm, dry winters and hot wet summers. Evidence of seroconversion was found in a recent study when animals were repeatedly sampled over a period of 24 months, which suggested that RVFV was endemic in the low-lying area in KZN (Chapter 2). A recent outbreak in goats was also reported in the Maputo Province ±100 km from the study area, in 2014 (Fafetine *et al*., 2016). Concurrently with the former study, mosquitoes were trapped over a period of 18 months to describe the population dynamics of potential RVFV vectors (Chapter 4). This chapter reports the detection, sequencing and phylogenetic analysis of RVFV, CHIKV and SINV from a single pool of *Ae.* (*Adm.*) *durbanensis* collected during that study.

5.2 MATERIALS AND METHODS

5.2.1 Mosquito trapping and processing

During the first year of the study, net traps were placed at two locations: one (Mpala) at the inlet of a stream into the Qotho pan in an open area of crops and grassland, with water present in the inlet for most of the year, and the other (Bumbe) at the perimeter of the Bumbe pan on the Pongolo River floodplain; the pan was dry throughout most of the study period, but permanent water was present in the river about 500 m away. Solid carbon dioxide was used as bait. Traps were placed and baited in the late afternoon just before sunset and emptied just before sunrise using handheld vacuums. All collections were placed on dry ice and examined microscopically for species identification, as discussed in Chapter 4. The total collection of 34,848 mosquitoes were pooled ($n \leq 50$) according to species and date and were homogenized using 2 mL homogenizing tubes (Anatech, city or country) in a Precellys 24 bead mill homogenizer (Anatech, Johannesburg) at 6000 rpm for 1 min

and centrifuged in a Eppendorf 5430 (Sigma Aldrich, Kempton Park). The samples were stored at -80°C until further processing.

5.2.2 Real-time reverse transcriptase polymerase chain reaction

Mosquito pools (n = 627) were selected including predominant species and known or suspected RVFV vectors (As discussed in Chapter 4). The total nucleic acid was extracted by using the magnetic bead technology of the MagMAX total Nucleic Acid Isolation Kit (Applied Biosystems), according to the instructions of the manufacturer.

A one-step reverse transcriptase real-time polymerase chain reaction (RT-PCR) (VetMax Plus OneStep RT-PCR Kit, Applied biosystems) was used to target the L segment of RVFV, according to the protocol of the manufacturer. The primers RVFL-2912FWDGG (5′-TGAAAATTCCTGAGACACATGG-3′) and primer RVFL2981REVAC (5′-ACTTCCTTGCATCATCTGATG3′) were used to amplify the initial diagnostic fragment as previously described (Bird *et al*., 2007).

A single RVFV RT-PCR positive mosquito pool homogenate (KZN 2017) was subjected to further screening, including an attempted virus isolation in suckling mice. After intracerebral inoculation, the mice died 2.5 days post inoculation. Brain material (MB1) was collected and used to inoculate Vero cells (MB1-Vero1).

The original sample and the MB1 and MB1-Vero1 passages were screened using a Real Star real-time reverse transcriptase polymerase chain reaction (rtRT-PCR) assay for CHIKV (Version 2.0). The samples were also screened for alphavirus (Giry *et al*., 2017). An unbiased sequencing approach was followed to sequence RNA extracted from the original mosquito homogenate, MB1, and MB1-Vero1.

The mosquito homogenate tested positive for RVFV, Chikungunya and *Alphavirus*. The original mosquito homogenate, the mouse brain passage and the Vero passage were sequenced using Sanger sequencing. Sanger sequencing of the amplified product was performed using BigDye Terminator Cycle Sequencing Ready Reaction Mix v3.1 (Applied Biosystems) followed by purification with BigDye XTerminator™ Purification Kit (Applied Biosystems) according to the manufacturer's instructions. The

sequences were compared to sequences on Genbank for CHIKV (Table 5.1), SINV (Table 5.2) and RFVF (Table 5.3).

5.2.3 Phylogenetic analysis

Rift Valley fever virus: The nucleic acid from 100 ul of the mosquito homogenate was extracted using the Biomeme M1 Sample Prep Cartridge Kit (Biomeme, Inc.). Presence of RVFV RNA was verified using the L and M primer/probes described in (Wilson *et al*., 2013) adapted to the Franklin Real-time PCR Thermocycler (Biomeme, Inc.). The sample was then sequenced using a targeted PCR sequencing approach modified from Bird *et al*., 2017. The sequencing library was prepared using a cDNA-PCR sequencing Kit (SQK-PCS108) with 15 cycles as described by the manufacturer (Oxford Nanopore Technologies) and sequenced on the Minion. The data were assembled after trimming and mapped to reference (KenO6 128B-1; KX096938) using CLC Genomic Workbench (ver. 12.0.1) (Qiagen Inc.). The consensus sequence was aligned to all available RVFV sequences. The alignment was trimmed to those portions where all sequence data was available. The aligned and trimmed L sequence data was export to Geneious version (10.2.3) and realigned using ClustalW, and the maximum likelihood trees were constructed using PhyML. The genetic distances were calculated using the Tamura-Nei method. Reliability patterns were assessed by a bootstrap analysis of 500 replications.

Table 5.1: Rift Valley fever virus L segment sequences with accession numbers used in this study.

Accession #	Isolate #/year	Location	Host	Reference
DQ375395	OS-8/87	Mauritania	*Homo sapiens*	Bird *et al*., 2007
DQ375396	OS-3/87	Mauritania	*Homo sapiens*	Bird *et al*., 2007
DQ375398	OS-1/87	Mauritania	*Homo sapiens*	Bird *et al*., 2007
DQ375400	Keya00523/98	Kenya	*Homo sapiens*	Bird *et al*., 2007
DQ375401	Saudi 10911/00	Saudi Arabia	*Homo sapiens*	Bird *et al*., 2007
DQ375402	Kenya 21445/83	Kenya Ruitu	Ae mcintoshi	Bird *et al*., 2007

DQ375408	ZH-501-777	Egypt Shaqiya	Plaque from ZH501	Bird *et al*., 200
DQ375413	2250/74	Zimbabwe Beatrce	Bovine foetus	Bird *et al*., 2007
DQ375414	MgH824/79	Madagascar	*Homo sapiens*	Bird *et al*., 2007
DQ375418	1260/78	Zimbabwe Salisbury	Bovine foetus	Bird *et al*., 2007
DQ375419	Zinga67	Central African Republic	*Homo sapiens*	Bird *et al*., 2007
DQ375421	ANK-6087/74	Guinea	Micropterud Pusillus	Bird *et al*., 2007
DQ375422	Hv-B375/85	Central African Republic Mbaki	*Homo sapiens*	Bird *et al*., 2007
DQ375423	CAR-R1622/85	Central African Republic Bangui	*Homo sapiens*	Bird *et al*., 2007
DQ375424	1853/78	Zimbabwe Sinoia	Bovine foetus	Bird *et al*., 2007
DQ375425	73HB1230	Central African Republic	*Homo sapiens*	Bird *et al*., 2007
DQ375426	730/70	Zimbabwe Salisbury	Bovine foetus	Bird *et al*., 2007
DQ375427	KenyaIB8/65	Kenya	Bovine	Bird *et al*., 2007
DQ375428	SA-75	Randfontein SA	*Homo sapiens*	Bird *et al*., 2007
DQ375429	Entebbe/44	Uganda	Mosquito	Bird *et al*., 2007
DQ375430	Smithburn	Uganda	Derived from Entebbe	Bird *et al*., 2007
DQ375431	Kenya57/51	Kenya	Ovine	Bird *et al*., 2007
DQ375432	2373/74	Zimbabwe Salisbury	Bovine foetus	Bird *et al*., 2007
DQ375433	SA-51	Boshof SA	Ovine	Bird *et al*., 2007
DQ375434	2267/74	Zimbabwe Sinoia	Bovine	Bird *et al*., 2007
EU574004	4194/2007	Kenya Kiambu	Bovine	Bird *et al*., 2008
EU574007	2007003081	Kenya Mbeere	Bovine	Bird *et al*., 2008
EU574009	2007002482	Kenya Thika	Bovine	Bird *et al*., 2008
EU574014	2007002060	Kenya Nairobi	Bovine	Bird *et al*., 2008
EU574014	1811/2007	Kenya Garissa	Caprine	Bird *et al*., 2008
EU574015	1809/2007	Kenya Garissa	Caprine	Bird *et al*., 2008
EU574017	1564/2007	Kenya Marunga	Bovine	Bird *et al*., 2008
EU574019	1292/2007	Kenya Meru South	Bovine	Bird *et al*., 2008
EU574020	1107/2007	Kenya Thika district	Bovine	Bird *et al*., 2008
EU574028	0222/2007	Kenya Maragua	Bovine	Bird *et al*., 2008
HE687304	099/2008	Mayotte	*Homo sapiens*	Cêtre-Sossah *et al*., 2012
HE687305	101/2008	Mayotte	*Homo sapiens*	Cêtre-Sossah *et al*., 2012
HM586953	Ken/Gar004/06	Kenya Fafi Jarajila	*Homo sapiens*	Nderitu *et al*., 201

HM586954	Ken/Gar008/06	Kenya Fafi Jarajila	*Homo sapiens*	Nderitu *et al*., 2010
HM586955	Ken/Kil-006/07	Kenya Bahari	*Homo sapiens*	Nderitu *et al*., 2011
HM586956	KenMal-032/07	Kenya	*Homo sapiens*	Nderitu *et al*., 2011
HM586957	Ken/Bar-032/07	Kenya Ichamus	*Homo sapiens*	Nderitu *et al*., 2011
HM586958	Ken/Bar-035/07	Kenya Kiserian	*Homo sapiens*	Nderitu *et al*., 2011
HM586959	Tan/Tan-001/07	Tanzania Tanga	*Homo sapiens*	Nderitu *et al*., 2011
HM586960	Tan/Tan-002/07	Tanzania Dodoma	Human serum	Nderitu *et al*., 2011
HM586961	Ken/GarMsq131B04/06	Kenya Fafi Jarajila	Aedes mosquito	Nderitu *et al*., 2011
HM586962	Ken/Klf-Msq091/07	Kenya Bahari	Aedes mosquito	Nderitu *et al*., 2011
JF311368	0212/08	Madegascar Taolagnaro	*Homo sapiens*	Carroll *et al*., 2011
JF311372	S97-41/1991	Madagascar: Antananarivo	*Homo sapiens*	Carroll *et al*., 2011
JF311373	ZF-06/1991	Madagascar: Antananarivo	Bovine	Carroll *et al*., 2011
JF311374	0406/08	Madegascar Miarinarivo	Bovine	Carroll *et al*., 2011
JF326186	23/2004	Kenya	*Homo sapiens*	Bird and Nichol, 2012
JF326189	383/2007	Tanzania	*Homo sapiens*	Bird and Nichol, 2012
JF326190	324/2007	Tanzania	*Homo sapiens*	Bird and Nichol, 2012
JQ820483	Sudan 2V-2010	Sudan: White Nile state	*Homo sapiens*	Aradaib *et al*., 2013
JQ820484	Sudan 86-2010	Sudan: Gezira state	*Homo sapiens*	Aradaib *et al*., 2013
JQ820485	Sudan 85-2010	Sudan: Gezira state	*Homo sapiens*	Aradaib *et al*., 2013
JQ820486	Sudan 28-2010	Sudan: Gezira state	*Homo sapiens*	Aradaib *et al*., 2013
KU167027	Lunyo	Uganda	Mosquito	Lumey *et al* 2016
KU978780	Ken 523/98	Kenya	*Homo sapiens*	Rossie *et al*., 2017
KX096938	Kenya-128b-15	Kenya Garissa	*Ae. ochraceus*	Shivanna *et al*., 2016
KX096941	SA01-1322/01	Saudi Arabia	*Ae. vexan arabiensis*	Shivanna *et al*., 2016
KX944849	M06/10	South Africa	Bovine	Maluleke *et al*., 2019
KX944851	M127/09	South Africa	Bovine	Maluleke *et al*., 2019
KX944854	M1955	South Africa	Mosquito	Maluleke *et al*., 2019
KX944855	M1975Bov	Not available	Bovine	Maluleke *et al*., 2019
KX944858	M25/10	South Africa	Ovine	Maluleke *et al*., 2019
KX944860	M260/09	South Africa	Bovine	Maluleke *et al*., 2019
KX944863	M37/08	Hoedspruit (LP) SA	Buffalo calf	Maluleke *et al*., 2019
KX944864	M39/08	Nelspruit (MP) SA	Buffalo calf	Maluleke *et al*., 2019
KX944864	M47/08	South Africa	Bovine	Maluleke *et al*., 2019
KX944866	M48/08	Madagascar	Bovine	Maluleke *et al*., 2019
MG273455	Kenya 90058/	Kenya		Ikegami *et al*., 2017

MG273458	OS7/	Mauritania		Ikegami *et al*., 2017
MG953421	201601502	Uganda	*Homo sapiens*	Shoemaker *et al*., 2019
MG953422	201601292	Uganda	*Homo sapiens*	Shoemaker *et al*., 2019
MH175203	CFIA-UAP	Manitoba	*Homo sapiens*	Kroeker *et al*., 2018

Chikungunya virus: The sequence obtained from KZN 2017 virus isolate was assembled to the NCBI reference sequence: NP_690588 (Khan *et al*., 2002). The sequences were trimmed to a final size of 3416 bp of the CHIKV from bp 6762 to 10179. Multiple sequence alignments were created using ClustalW ® incorporated into Geneious®11.1.2. PhyML version 7.2.8 incorporated into Geneious was used to construct a maximum likelihood phylogram with 1000 bootstrap replicates to estimate the branch support.

Table 5.2: Chikungunya virus, complete genome sequences with accession numbers used in this study.

Accession #	Isolate/year	Collection	Host	Reference
EU372006	DRDE/2007	India - Kerala state	*Homo sapiens*	Unpublished
FR717336	IMTSSA6424S/05	France- La Reunion	*Homo sapiens*	Couderc *et al*., 2012
FR717337	IMTSSA6424C/05	France- La Reunion	*Homo sapiens*	Couderc *et al*., 2012
GU013528	LK(PB)chik3408/08	Sri Lanka	*Homo sapiens*	Hapuarachchi *et al*., 2010
HM045792	Vereeniging/1956	South Africa - Vereeniging	*Homo sapiens*	Volk *et al*., 2010
HM045793	CAR256	Central African Republic		Volk *et al*., 2010
HM045795	SAH2123/1976	South Africa	*Homo sapiens*	Volk *et al*., 2010
HM045796	CO392/1995	Thailand	*Homo sapiens*	Volk *et al*., 2010
HM045799	SL-CR 3/2007	Sri Lanka	*Homo sapiens*	
HM045802	K0146/1995	Thailand		Volk *et al*., 2010
HM045803	I-634029/1963	India	*Homo sapiens*	Volk *et al*., 2010
HM045805	AR18211/1976	South Africa	*Ae. furcifer*	Volk *et al*., 2010
HM045806	ALSA-1/1986	India	*Ae. albopictus*	Volk *et al*., 2010
HM045808	3412-78/1978	Thailand	*Homo sapiens*	Volk *et al*., 2010
HM045809	LSFS/1960	Democratic Republic of the Congo	*Homo sapiens*	Volk *et al*., 2010
HM045810	TH35/1958	Thailand	*Homo sapiens*	Volk *et al*., 2010

HM045811	Ross low psg/1953	Tanzania	*Homo sapiens*	Volk *et al*., 2010
HQ456253	Com125/2005	Grande Comoros, Comoros	*Ae. Aegypti*	Njenga *et al*., 2008
HQ456254	KPA15/2005	Mombasa -Kenya	*Homo sapiens*	Njenga *et al*., 2008
HQ456255	Lamu33/2004	Lamu island - Kenya	*Homo sapiens*	Njenga *et al*., 2008
KJ796845	RGCB729/2009	India - Kerala state	Serum	Tsetsarkin *et al*., 2011
KJ796848	CK80/2008	Thailand	*Homo sapiens*	Tsetsarkin *et al*., 2011
KP003808	MADOPY1/2006	Madagascar	*Homo sapiens*	Moyen *et al*., 2014
KY703946	CHIKV/1953	Nicaragua: Managua	*Homo sapiens*	Unpublished
LN898108	M109/2014	Martinique	*Homo sapiens*	Stapleford *et al*., 2016
LN898109	M110/2014	Martinique	*Homo sapiens*	Stapleford *et al*., 2016
LN898110	G106/2014	Guadeloupe	*Homo sapiens*	Stapleford *et al*., 2016
LN898111	G107/2014	Guadeloupe	*Homo sapiens*	Stapleford *et al*., 2016
MF499120	hk02/2016	Hong Kong	*Homo sapiens*	Narang *et al*., 2019
MF773559	Samoa/2014	Samoa	*Homo sapiens*	Pyke *et al*., 2018
MF773560	Caribbean/2014	Caribbean	*Homo sapiens*	Pyke *et al*., 2018
MF773561	Bali 2011	Bali - Indonesia	*Homo sapiens*	Pyke *et al*., 2018
MF773562	Kiribati/2015	Kiribati	*Homo sapiens*	Pyke *et al*., 2018
MF773563	phil 2014	Philippines	*Homo sapiens*	Pyke *et al*., 2018
MF773564	Phil 2016	Philippines	*Homo sapiens*	Pyke *et al*., 2018
MF773565	ET2010	Timor- Leste	*Homo sapiens*	Pyke *et al*., 2018
MF773566	Bangladesh/2017	Bangladesh	*Homo sapiens*	Pyke *et al*., 201
MF773567	Borneo/2011	Borneo	*Homo sapiens*	Pyke *et al*., 2018
MF773568	Malaysia/2008	Malaysia	*Homo sapiens*	Pyke *et al*., 2018
MH229986	6113879/2006	Mauritius	*Homo sapiens*	Unpublished
MH423801	063_2016_S44	Kenya - Mandera	*Homo sapiens*	Berry *et al*., 2018
MH423802	068_2016_S55	Kenya - Mandera	*Homo sapiens*	Berry *et al*., 2018
MH754507	Lazio-ISS-1/2017	Italy - Lazio	*Ae. Albopictus*	Lindh *et al*., 2018

Sindbis virus: The sequence obtained from the KZN 2017 virus isolate (MB1-Ver01) was assembled to the NCBI reference sequence: NC_001547 (Strauss *et al*., 1984). The whole genome sequences were trimmed to a final size of 11,412 bp of the SINV. Multiple sequence alignments were created using ClustalW ® incorporated into Geneious®11.1.2. PhyML version 7.2.8 that is incorporated into Geneious was used to construct a Maximum likelihood phylogram with 1000 bootstrap replicates to estimate the branch support.

Table 5.3: Sindbis virus, complete genome sequences with accession numbers used in this study

Accession #	Isolate #/year	Location	Host	Reference
AF103734	YN87448/1992	China Yunnan	*Homo sapiens*	Liang *et al*., 2000
AF429428	SW6562	South West Australia		Saleh *et al*., 2003
GU361116	Isolate 5,3/2009	Germany	*An. maculipennis*	Jöst *et al*., 2010
GU361118.2	isolate 28.9/2009	Germany	*Cx. torrentium*	Jöst *et al*., 2010
J02363	2000			Ou *et al*., 1981
JQ771794	Ilomantsi-2002A/02	Ilomantsi, Finland	*Homo sapiens*	Sane *et al*., 2012
JQ771795	Ilomantsi-200202	Ilomantsi, Finland	*Homo sapiens*	Sane *et al*., 2012
JQ771797	Johannes-2002	Finland	*Homo sapiens*	Sane *et al*., 2012
JQ771798	Kiihtelysvaara-2002	Kiihtelysvaara, Finland		Sane *et al*., 2012
JX570540	Berlin-2010A	Germany - Berlin	Corvus cornix	Eiden *et al*., 2014
KF737350	Lovanger/2013	Sweden, Lovanger,	*Culiseta morsitans*	Bergqvist *et al*., 2015
KY616985	BONI_584_KENYA_2013	Kenya: Boni forest	*Ae. tricholabis*	Unpublished
KY616986	KSM_1008_KENYA_2007	Kenya: Kisumu	*Culex* spp.	Unpublished
KY616987	BONI_566_KENYA_2013	Kenya: Boni forest	*Ae. ochraceus*	Unpublished
KY616988	NVS_305	Naivasha - Kenya	*Cx. vansomereni*	Unpublished
MF543016	Giessen_2016_A	Germany	Columba palumbus	Unpublished
MF589985	BNI-10865/2016	Heidelberg, Ochsenkopf	*Cx. pipiens*	Jansen *et al*., 2018
MG182396	Isolate 18953,1	Australia: Charleville	*Cx. annulirostris*	Pickering *et al*., 2019
MG495620	BNI-CuliMoCuliMo543/2016	Germany	*Cx. pipiens*	Unpublished
MG679373	Altay	Russia: Altay krai		Belyaev *et al*., 2018
MG679374	LEIV-65A	Azerbaijan		Belyaev *et al*., 2018
MG679375	Stavropol	Russia: Stavropol		Belyaev *et al*., 2018
MG679376	F-720/1990	Armenia	*Bubulcus ibis*	Belyaev *et al*., 2018
MG679377	LEIV-Ast03-1-839/2003	Russia: Astrakhan		Belyaev *et al*., 2018
MG679378	LEIV-Ast03-1-844/2003	Russia: Astrakhan		Belyaev *et al*., 2018
MG679380	Tatarstan	Russia: Tatarstan		Belyaev *et al*., 2018
MG779533	15Z03121/2015	Germany	*Cx. torrentium*	Scheuch *et al*., 2018
MG779534	H7/163	Germany	*Cx. pipiens*	Scheuch *et al*., 2018
MH212167	ArB7761/1977	Central African Republic-Bonzo	*Cx. cinereus*	Ouilibona *et al*., 2018
MH229928	YN_222/2013	China: Yunnan Province	Midge	Wang *et al*., 2011
U38304	Girdwood SA	Russe; *et al*., 1996		(Simpson *et al*., 1996)
U38305	S.A.AR86/1954	South Africa		(Simpson *et al*., 1996)

5.3 RESULTS

Total number of 627 pools of mosquito homogenates were screened for RVFV using RT-PCR, one pool that consisted of only unfed female *Ae. durbanensis* mosquitoes caught in March 2017 at Mpala, KZN, tested positive for RVFV. The sample was labelled KZN 2017 isolate and sent to the National Institute for Communicable Diseases (Sandringham, Johannesburg, South Africa) for further processing. The mosquito homogenate tested positive for RVFV, Chikungunya and an *Alphavirus*.

Rift Valley fever virus

The RVFV isolated in this study (KZN 2017) clustered with two isolates from South Africa (Fig. 5.1): one obtained in 1951 at Boshof in the Free State Province from a clinically infected sheep, and the other obtained in 1955 from an *Ae. (Neo.) circumluteolus* mosquito in northern KZN at the Simbu pan, about 20 km from the collection site for this study. Notably, the isolate in this study was not closely related to other isolates obtained from eastern South Africa (Lineage C), the recent small outbreak in southern Mozambique (Lineage C), or the virus responsible for the most recent large outbreak in South Africa (Lineage H).

Chikungunya virus

The partial CHIKV sequence clustered with two strains previously isolated in South Africa, within the ECSA lineage (Fig. 5.2). The one isolate was obtained in 1976 from *Ae. (Dic.) furcifer* from a small rural outbreak in the wooded savanna region of the eastern Transvaal (Limpopo Province) of South Africa (Jupp and McIntosh, 1990; McIntosh and Jupp, 1977). The other isolate was obtained from a human patient during the same outbreak. The patients were all from rural areas and two resided on the same farm where the isolate from the mosquito were gained. No information is available to conclude whether the isolate from the human patient and the isolate from the mosquito were collected from the same farm (McIntosh and Jupp, 1977).

Sindbis virus

The partial SINV sequence clustered with two isolates; the first (SA.AR86) was isolated in 1954 from a pool of *Culex* mosquitoes collected near Johannesburg South Africa (Weinbren *et al*., 1956). The other isolate (YN87448) was obtained from a Chinese patient suffering from a febrile disease, in Yunnan in China (Zhou *et al*., 1999) which has been classified as Sindbis virus closely related to Girdwood South African strains (Liang *et al*., 2000) (Fig. 5.3).

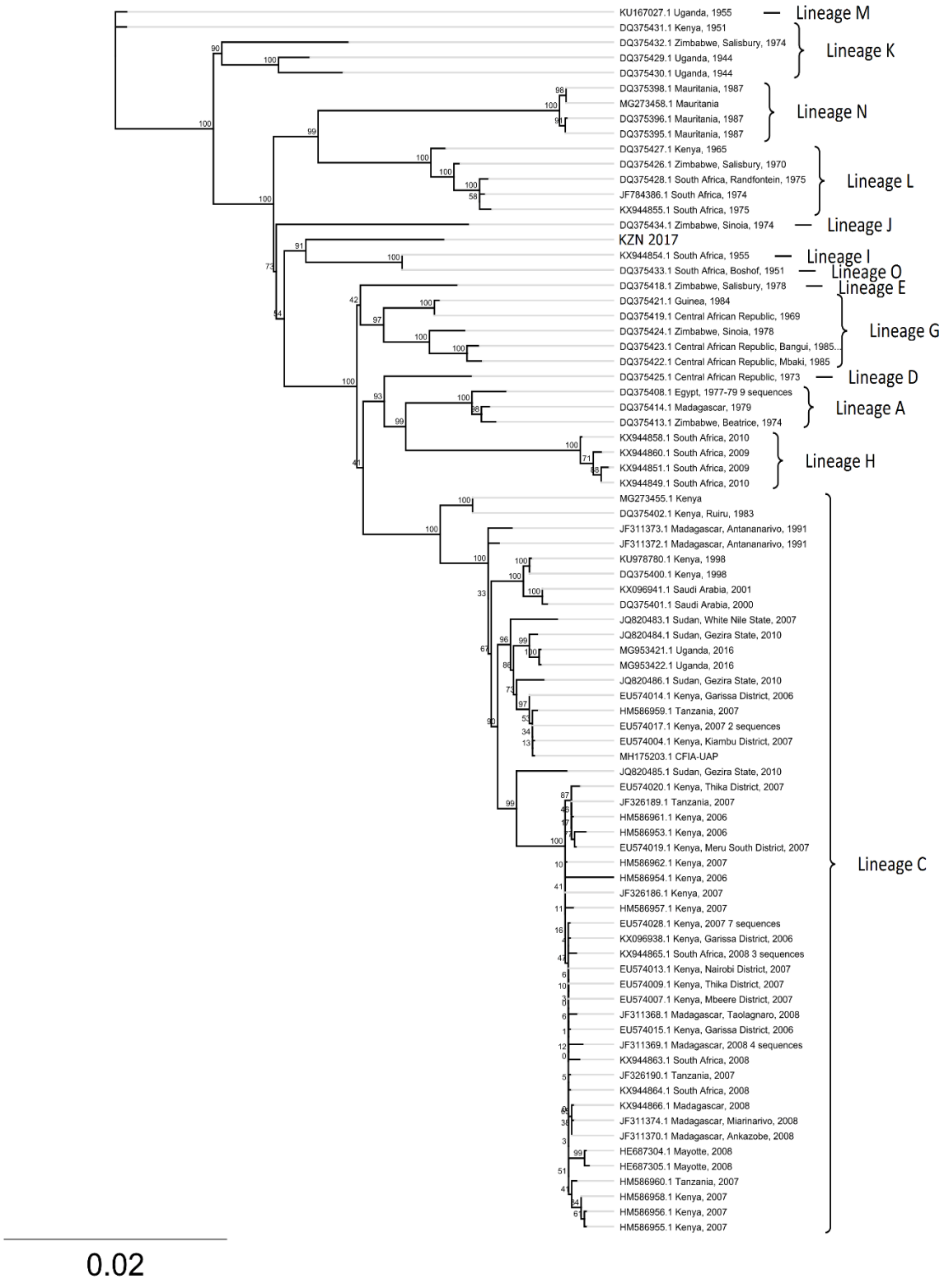


Figure 5.1: Maximum likelihood phylogenetic tree indicating the genetic relationship of the partial genome of Rift Valley fever virus compared to other sequences from GenBank, presented in a phylogram with 1000 bootstrap replicates to estimate branch support. The scale bar indicates genetic distance (mutations per site). The lineages are according to Grobbelaar *et al.* (2013)

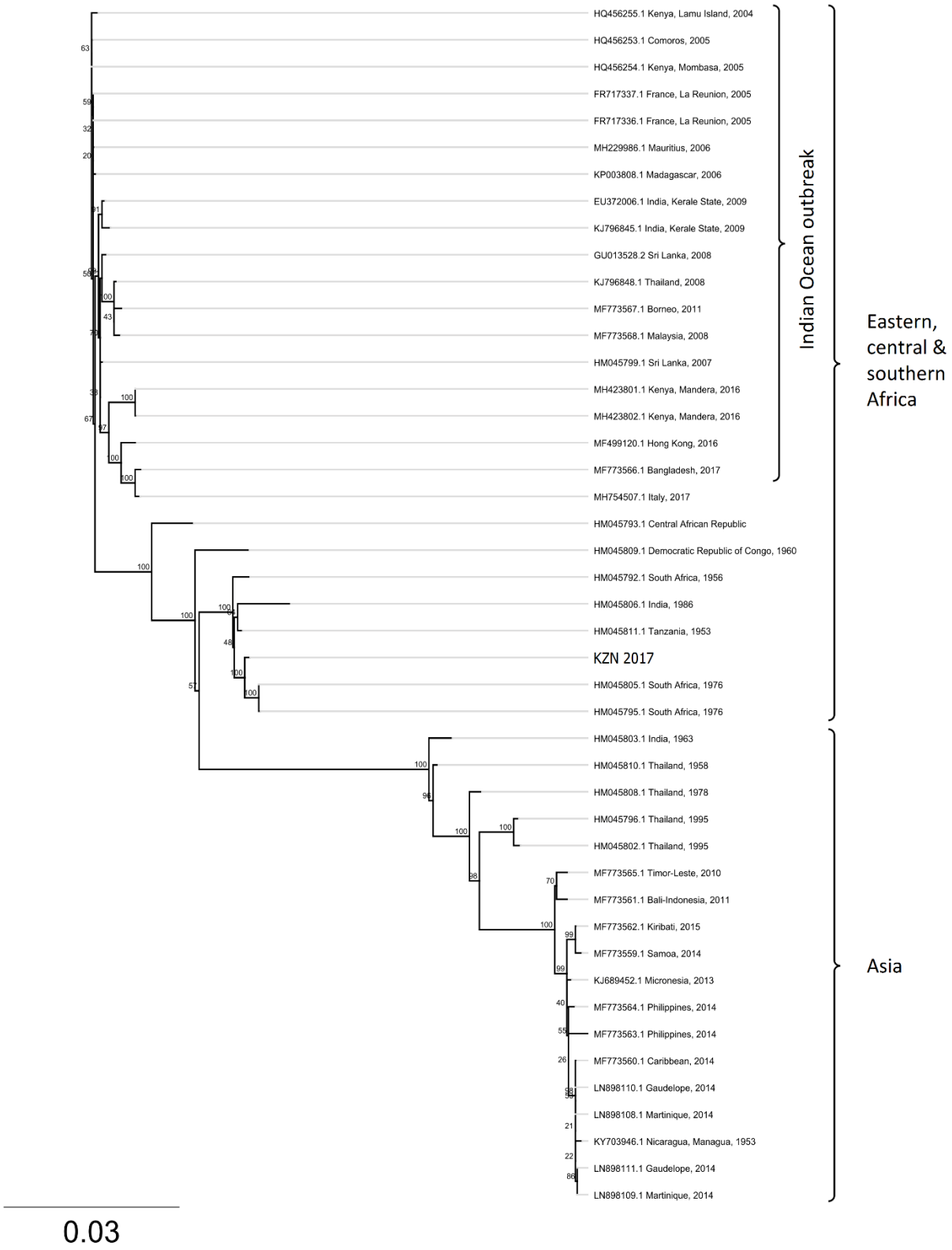


Figure 5.2: Maximum likelihood phylogenetic tree indicating the genetic relationship of the partial genome of Chikungunya virus compared to other sequences from GenBank, presented in a phylogram with 1000 bootstrap replicates to estimate branch support. The side bar indicates genetic distances.

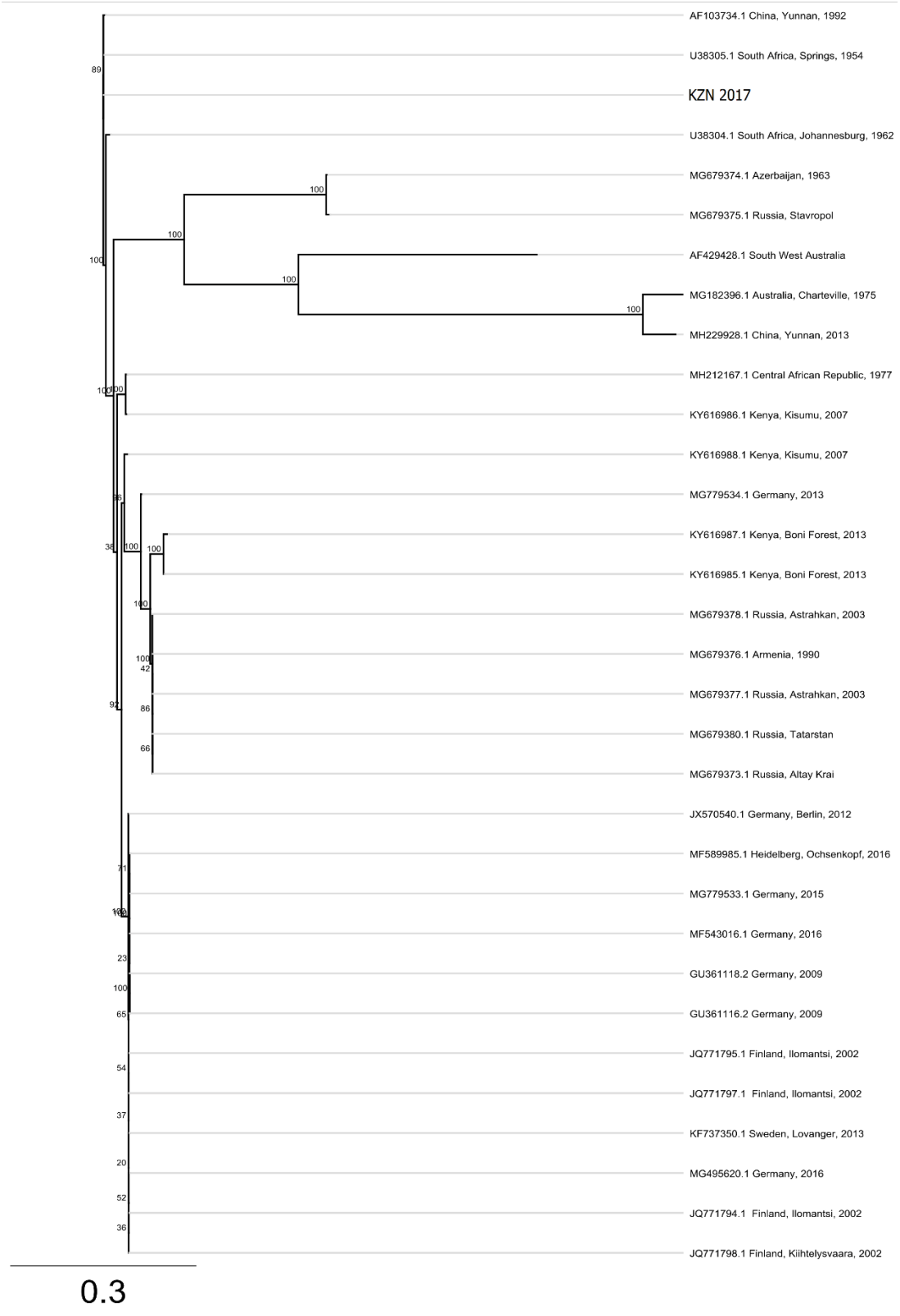


Figure 5.3: Maximum likelihood phylogenetic tree indicating the genetic relationship of the whole genome of Sindbis virus compared to other sequences from GenBank, presented in a phylogram with 1000 bootstrap replicates to estimate branch support. The side bar indicates genetic distances.

5.4 DISCUSSION

This was part of a larger study of RVFV epidemiology (van den Bergh *et al*., 2019), and reports the simultaneous detection of multiple zoonotic arboviruses (RVFV, CHIKV and SINV) in a single pool of unfed *Ae. (Adm.) durbanensis* mosquitoes collected in northern KZN. No simultaneous detection of viruses in a single field-collected mosquito pool has been published, however an experimental study demonstrated that *Ae. (Stg.) aegypti* mosquitoes can replicate CHIKV, Dengue and Zika viruses and support coinfection with all three viruses, resulting in an ability to transmit all combinations of these viruses simultaneously (Rückert *et al*., 2017).

The mosquitoes were collected in an area where RVFV outbreaks were reported before. Limited outbreaks were reported near Empangeni and Hluhluwe, South Africa and Mozambique and cattle were the only species that were affected during this outbreak (McIntosh, 1972). No clinical disease observations were reported in the coastal lowlands of Natal closer to the border of Mozambique when high numbers of *Ae. (Adm.) durbanensis* were reported. Multiple RVFV isolates were obtained in 1953 in the Luckhoff District in the Orange Free State Province from *Cx. theileri* and *Ae. (Och.) caballus (*Gear *et al.*, 1955*)* which is different from the species collected in 1955 where common species found in Mozambique were *Ae. (Neo.) circumluteolus, Cx. (Cux.) thalassius, Cx. (Cux.) poicilipes and An. (Ano.) coustani*. Rift Valley fever virus was isolated twice in 1955 from *Ae. (Neo.) circumluteolus* which at that time was a dominant species in the coastal zone of the northern Natal (Kokernot *et al*., 1957a). However, it was not detected in subsequent screening of over 340,000 mosquitoes in that area during 1956-1968 (Worth *et al*., 1961, McIntosh *et al*., 1972). The sequence obtained from the mosquito homogenate in this study was closely related to the isolate from 1955, suggesting that the virus has been circulating in the area for more than 60 years. The sequence obtained is also very closely related to an isolate obtained in 1951 from the first known outbreak in South Africa, Transvaal State Province. This may indicate that the movement of an infected animal from KZN, an endemic area, to an area with more susceptible ruminants like sheep might have initiated the first large outbreak in SA. The possibility that the movement of infected animals from KZN to the

interior parts of the country might be a cause of outbreaks when the environment becomes favourable, should be investigated.

The RVFV sequence obtained from the mosquito homogenate was not closely related to Lineage C, the lineage responsible for outbreaks in Komatipoort, South Africa in 2008 (Pienaar and Thompson, 2013) and the outbreak in 2014 in Maputo Province, Mozambique (Fafetine *et al*., 2016). This was the most likely lineage expected in the area. Since this was not the case it may imply that that there might be more than one lineage circulating in the area. This was the case in Kenya during 2006-2007. Multiple lineages including B, C, K and L circulated in parallel in the area (Grobbelaar *et al*., 2011, Ikegami, 2012). The same situation occurred in Zimbabwe where isolates suggested co-circulation of multiple strains in the area around Harare (Swanepoel *et al*., 1979, Ikegami, 2012).

Several other arboviruses have been previously isolated from this area; in 1955, Spondweni virus was isolated from *Mansonia (Man.) uniformis,* while Pongola, Simbu and Wesselbron viruses were isolated from *Ae. (Neo.) circumluteolus* (Muspratt *et al*., 1957, Kokernot *et al*., 1957b, Worth *et al*., 1961, McIntosh *et al*., 1972). During the study we detected CHIKV and SINV. Chikungunya virus occurs infrequently in South Africa in the eastern Transvaal and the coastal northern Natal. Sixteen isolations of CHIKV have been obtained from *Ae. (Dic.) furcifer/taylori* group in the Transvaal on the wooded savanna area of South Africa (McIntosh and Jupp, 1977). Chikungunya virus is an arbovirus that is vectored by *Aedes* mosquitoes to humans and primates in tropical and sub-tropical regions of Africa and Asia. It causes a febrile disease with severe arthralgia and a rash (Adebajo, 1996, Deller and Russell, 1967, Ligon, 2006). Sindbis virus causes infections in humans mainly during the summer months and is widely dispersed in South Africa in Gauteng, Free State and the Limpopo Province (Jupp and NK, 1986). The main vectors have been identified as *Cx. (Cux.) univittatus* and *Cx. (Cux.) neavei* but several other species have been proven competent to transmit the disease (Jupp, 2005). During 1956-1968, SINV was isolated several times from *Ae. (Neo.) circumluteolus*, *Ae. (Adm.) cumminsii*, *Cx. (Cux.) neavei* and *Ma. (Man.) africana* collected in Ndumo Game Reserve (Worth et al 1961; McIntosh et al. 1972).

In South Africa CHIKV is restricted to the eastern Transvaal lowveld (Limpopo Province) and the coastal northern Natal (KZNProvince). An outbreak was detected serologically among vervet monkeys (*Chlorocebus pygerythrus* formerly included in *Cercopthecus aethiops*) in Ddumo Game Reserve in 1964. However, CHIKV was not detected in more than 340,000 mosquitoes collected in the area during 1956-1968 (McIntosh, 1970). Another outbreak was reported in Mica in the north-eastern Transvaal in 1976 (McIntosh and Jupp, 1977). The primary vector of the virus is *Ae. (Dic.) furcifer* but *Ae. (Stg.) aegypti*, *Ae. (Stg.) albopictus*, *Ae. (Fre.) vittatus* and *Ae. (Fin.) fulgens* have shown to be competent vectors in laboratory experiments (Jupp and McIntosh, 1990). In this study the virus was isolated from *Ae. (Adm.) durbanensis* for the first time. This might be because, since 1968, little work has been done on mosquitoes in the area, other than *Anopheles* the primary vector of malaria. But this might also be because of the methods used to collect the mosquitoes screened for CHIKV. Because the virus affects humans and primates the collections are normally based close to trees or in treetops using humans and monkeys as bait (Jupp and McIntosh, 1990). This was not the case for this study as our aim was to identify potential vectors of RVFV. None of the above-mentioned studies reported that they collected *Ae. (Adm.) durbanensis* and this might be due to the fact that this mosquito species prefers to feed on cattle and goats rather than humans (Sharp *et al*., 1988). It is evident from these results that some viruses might be overlooked, either because studies may only screen for single viruses. Another possibility might be because the collection methods are aimed at suspected vectors, while other unknown vectors might not be collected at all.

Symptoms in humans resulting from infection with these viruses (CHIKV, RVFV and SINV) are non-specific and mild in the majority of cases. It is therefore likely that the majority of cases are not reported in rural areas where laboratory diagnostics are absent. The potential harm to human health is unknown, also since it is considered that RVFV infections in humans occur mainly due to direct contact with infected animal tissue and that infection via mosquito bites is less common in humans. The opposite appears to be true for infection with CHIKV. *Aedes (Dic.) furcifer* was the most prevalent mosquito in the 1976 a CHIKV outbreak in Mica, in the North eastern Transvaal, and was responsible for virus transmission between lower primates and

humans (McIntosh and Jupp, 1977). Other vectors that were competent to transmit the disease under laboratory conditions were *Ae. (Stg.) aegypti*, *Ae. (Fin.) fulgens* and *Ae. (Fre.) vittatus* (Jupp and McIntosh, 1990). To our knowledge no clinical cases of RVFV, CHIKV and SINV have ever been reported in this area, likely because these diseases present mostly as mild febrile diseases and may be misdiagnosed as malaria which is common in the area. A study done 100 km north of the study area in Maputo where 5% seroconversion to RVFV in febrile patients, indicate clinical disease in humans that the virus cause in the region but is overlooked or misdiagnosed as malaria (Gudo *et al*., 2016)

In conclusion the study reports simultaneous detection of multiple zoonotic arboviruses from a single mosquito pool. Further investigation is needed to determine whether *Ae. (Adm.) durbanensis* is a competent vector for any of the viruses isolated, to identify other vector species, and to assess the potential impact of RVFV, CHIKV and SINV on human health in the area.

REFERENCES

1. ADAMS, M. J., LEFKOWITZ, E. J., KING, A. M., HARRACH, B., HARRISON, R. L., KNOWLES, N. J., KROPINSKI, A. M., KRUPOVIC, M., KUHN, J. H. & MUSHEGIAN, A. R. 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Archives of Virology,* 162**,** 2505-2538.
2. ADEBAJO, A. O. 1996. Rheumatic manifestations of tropical diseases. *Current opinion in rheumatology,* 8**,** 85-89.
3. BERGQVIST, J., FORSMAN, O., LARSSON, P., NÄSLUND, J., LILJA, T., ENGDAHL, C., LINDSTRÖM, A., GYLFE, Å., AHLM, C. & EVANDER, M. 2015. Detection and isolation of Sindbis virus from mosquitoes captured during an outbreak in Sweden, 2013. *Vector-Borne and Zoonotic Diseases,* 15**,** 133-140.
4. BERRY, I. M., EYASE, F., POLLETT, S., KONONGOI, L. S., FIGUEROA, K., OFULA, V., KOKA, H., KOSKEI, E., NYUNJA, A. & MANCUSO, J. D. 2018. Recent outbreaks of chikungunya virus (CHIKV) in Africa and Asia are driven by a variant carrying mutations associated with increased fitness for Aedes aegypti. *BioRxiv***,** 373316.
5. BRUMMER-KORVENKONTIO, M., VAPALAHTI, O., KUUSISTO, P., SAIKKU, P., MANNI, T., KOSKELA, P., NYGREN, T., BRUMMER-KORVENKONTIO, H. & VAHERI, A. 2002. Epidemiology of Sindbis virus infections in Finland 1981–96: possible factors explaining a peculiar disease pattern. *Epidemiology & Infection,* 129**,** 335-345.
6. CAREY, D. E. 1971. Chikungunya and dengue: a case of mistaken identity? *Journal of the History of Medicine and Allied Sciences,* 26**,** 243-262.
7. CAUCHEMEZ, S., LEDRANS, M., POLETTO, C., QUENEL, P., DE VALK, H., COLIZZA, V. & BOELLE, P.-Y. 2014. Local and regional spread of chikungunya fever in the Americas. *Euro surveillance: bulletin Europeen sur les maladies transmissibles, European communicable disease bulletin,* 19**,** 20854.
8. CHARREL, R. N., DE LAMBALLERIE, X. & RAOULT, D. 2007. Chikungunya outbreaks-the globalization of vectorborne diseases. *New England Journal of Medicine,* 356**,** 769.
9. CHRETIEN, J.-P., ANYAMBA, A., BEDNO, S. A., BREIMAN, R. F., SANG, R., SERGON, K., POWERS, A. M., ONYANGO, C. O., SMALL, J. & TUCKER, C. J. 2007. Drought-associated chikungunya emergence along coastal East Africa. *The American Journal of Tropical Medicine and Hygiene,* 76**,** 405-407.
10. COUDERC, T., GANGNEUX, N., CHRÉTIEN, F., CARO, V., LE LUONG, T., DUCLOUX, B., TOLOU, H., LECUIT, M. & GRANDADAM, M. 2012. Chikungunya virus infection of corneal grafts. *The Journal of Infectious Diseases,* 206**,** 851-859.
11. DELLER, J. J. & RUSSELL, P. K. 1967. An analysis of fevers of unknown origin in American soldiers in Vietnam. *Annals of Internal Medicine,* 66**,** 1129-1143.
12. EIDEN, M., ZIEGLER, U., KELLER, M., MÜLLER, K., GRANZOW, H., JÖST, H., SCHMIDT-CHANASIT, J. & GROSCHUP, M. H. 2014. Isolation of Sindbis virus from a hooded crow in Germany. *Vector-Borne and Zoonotic Diseases,* 14**,** 220-222.
13. FAFETINE, J. M., COETZEE, P., MUBEMBA, B., NHAMBIRRE, O., NEVES, L., COETZER, J. A. & VENTER, E. H. 2016. Rift Valley fever outbreak in livestock, Mozambique, 2014. *Emerging Infectious Diseases,* 22**,** 2165.
14. FONTENILLE, D., TRAORE-LAMIZANA, M., DIALLO, M., THONNON, J., DIGOUTTE, J. & ZELLER, H. 1998. New vectors of Rift Valley fever in west Africa. *Emerging Infectious Diseases,* 4**,** 289.
15. GAD, A., FEINSOD, F., ALLAM, I., EISA, M., HASSAN, A., SOLIMAN, B. & SAAH, A. 1986. A possible route for the introduction of Rift Valley fever virus into Egypt during 1977. *The Journal of Tropical Medicine and Hygiene,* 89**,** 233-236.

16. GEAR, J., DE MEILLO, B., LE ROUX, A., KOFSKY, R., ROSE, R., STEYN, J., OLIFF, W. & SCHULZ, K. 1955. Rift valley fever in South Africa: A study of the 1953 outbreak in the Orange Free State, with special reference: To the vectors and possible reservoir hosts. *South African Medical Journal,* 29.
17. GEAR, J., DE MEILLON, B., MEASROCH, V. & DAVIS, D. 1951. Rift Valley fever in South Africa: 2: the occurrence of human cases in the Orange Free State, the North-Western Cape Province, the Western And Southern Transvaal: B: field and laboratory investigations. *African Journal of Health Professions Education,* 2**5,** 908-912.
18. GEAR, J. & REID, F. 1957. The Occurrence of a Dengue-Like Fever in the North-Eastern Transvaai. I. Clinical Features and Isolation of Virus. *South African Medical Journal,* 31**,** 253-7.
19. GIRY, C., ROQUEBERT, B., LI-PAT-YUEN, G., GASQUE, P. & JAFFAR-BANDJEE, M.-C. 2017. Improved detection of genus-specific Alphavirus using a generic TaqMan® assay. *BMC Microbiology,* 17**,** 164.
20. GRANDADAM, M., CARO, V., PLUMET, S., THIBERGE, J.-M., SOUARES, Y., FAILLOUX, A.-B., TOLOU, H. J., BUDELOT, M., COSSERAT, D. & LEPARC-GOFFART, I. 2011. Chikungunya virus, southeastern France. *Emerging Infectious Diseases,* 17**,** 910.
21. GROBBELAAR, A. A., WEYER, J., LEMAN, P. A., KEMP, A., PAWESKA, J. T. & SWANEPOEL, R. 2011. Molecular epidemiology of Rift Valley fever virus. *Emerging Infectious Diseases,* 17**,** 2270.
22. GUDO, E. S., PINTO, G., WEYER, J., LE ROUX, C., MANDLAZE, A., JOSÉ, A. F. & PAWESKA, J. T. 2016. Serological evidence of Rift Valley fever virus among acute febrile patients in Southern Mozambique during and after the 2013 heavy rainfall and flooding: implication for the management of febrile illness. *Virology Journal,* 13**,** 96.
23. HAPUARACHCHI, H., BANDARA, K., SUMANADASA, S., HAPUGODA, M., LAI, Y.-L., LEE, K.-S., TAN, L.-K., LIN, R. T., NG, L. F. & BUCHT, G. 2010. Re-emergence of Chikungunya virus in South-east Asia: virological evidence from Sri Lanka and Singapore. *Journal of General Virology,* 91**,** 1067-1076.
24. HOLLIDGE, B. S., GONZÁLEZ-SCARANO, F. & SOLDAN, S. S. 2010. Arboviral encephalitides: transmission, emergence, and pathogenesis. *Journal of Neuroimmune Pharmacology,* 5**,** 428-442.
25. HOMENAUTH, E., KAJEGUKA, D. & KULKARNI, M. A. 2017. Principal component analysis of socioeconomic factors and their association with malaria and arbovirus risk in Tanzania: a sensitivity analysis. *Journal of Epidemiology and Community Health,* 71**,** 1046-1051.
26. HOOGSTRAAL, H., MEEGAN, J. M., KHALIL, G. M. & ADHAM, F. K. 1979. The Rift Valley fever epizootic in Egypt 1977–1978 2. Ecological and entomological studies. *Transactions of the Royal Society of tropical Medicine and Hygiene,* 73**,** 624-629.
27. IKEGAMI, T. 2012. Molecular biology and genetic diversity of Rift Valley fever virus. *Antiviral Research,* 95**,** 293-310.
28. JÖST, H., BIALONSKI, A., STORCH, V., GÜNTHER, S., BECKER, N. & SCHMIDT-CHANASIT, J. 2010. Isolation and phylogenetic analysis of Sindbis viruses from mosquitoes in Germany. *Journal of Clinical Microbiology,* 48**,** 1900-1903.
29. JUPP, G. & NK, T. 1986. Sindbis and West Nile virus infections in the Witwatersrand-Pretoria region. *South African Medical Journal,* 70**,** 218-220.
30. JUPP, P. 1988. Chikungunya virus disease. *Arbovirus: Epidemiology and Ecology***,** 137.
31. JUPP, P. 2005. Mosquitoes as vectors of human disease in South Africa. *South African Family Practice,* 47**,** 68-72.
32. JUPP, P. & MCINTOSH, B. 1990. Aedes furcifer and other mosquitoes as vectors of chikungunya virus at Mica, northeastern Transvaal, South Africa. *Journal of the American Mosquito Control Association,* 6**,** 415-20.

33. KHAN, A. H., MORITA, K., DEL CARMEN PARQUET, M., HASEBE, F., MATHENGE, E. G. & IGARASHI, A. 2002. Complete nucleotide sequence of chikungunya virus and evidence for an internal polyadenylation site. *Journal of General Virology,* 83**,** 3075-3084.
34. KOKERNOT, R., HEYMANN, C., MUSPRATT, J. & WOLSTENHOLME, B. 1957a. Studies on arthropod-borne viruses of Tongaland. V. Isolation of Bunyamwera and Rift Valley Fever viruses from mosquitoes. *South African Journal of Medical Sciences,* 22**,** 71-80.
35. KOKERNOT, R., SMITHBURN, K., MUSPRATT, J. & HODGSON, B. 1957b. Studies on arthropod-borne viruses of Tongaland. VIII. Spondweni virus, an agent previously unknown, isolated from Taeniorhynchus (Mansonioides) uniformis Theo. *South African Journal of Medical Sciences,* 22**,** 103-112.
36. KROEKER, A., SMID, V., EMBURY-HYATT, C., MOFFAT, E., COLLIGNON, B., LUNG, O., LINDSAY, R. & WEINGARTL, H. 2018. RVFV Infection in Goats by Different Routes of Inoculation. *Viruses,* 10**,** 709.
37. KURKELA, S., MANNI, T., MYLLYNEN, J., VAHERI, A. & VAPALAHTI, O. 2005. Clinical and laboratory manifestations of Sindbis virus infection: prospective study, Finland, 2002–2003. *The Journal of infectious diseases,* 191**,** 1820-1829.
38. LANGSJOEN, R. M., HALLER, S. L., ROY, C. J., VINET-OLIPHANT, H., BERGREN, N. A., ERASMUS, J. H., LIVENGOOD, J. A., POWELL, T. D., WEAVER, S. C. & ROSSI, S. L. 2018. Chikungunya virus strains show lineage-specific variations in virulence and cross-protective ability in murine and nonhuman primate models. *MBio,* 9**,** e02449-17.
39. LEPARC-GOFFART, I., NOUGAIREDE, A., CASSADOU, S., PRAT, C. & DE LAMBALLERIE, X. 2014. Chikungunya in the Americas. *The Lancet,* 383**,** 514.
40. LIANG, G.-D., LI, L., ZHOU, G.-L., FU, S.-H., LI, Q.-P., LI, F.-S., HE, H.-H., JIN, Q., HE, Y. & CHEN, B.-Q. 2000. Isolation and complete nucleotide sequence of a Chinese Sindbis-like virus. *Journal of General Virology,* 81**,** 1347-1351.
41. LIGON, B. L. Reemergence of an unusual disease: the chikungunya epidemic. Seminars in pediatric infectious diseases, 2006. *Elsevier*, 99-104.
42. LINDH, E., ARGENTINI, C., REMOLI, M. E., FORTUNA, C., FAGGIONI, G., BENEDETTI, E., AMENDOLA, A., MARSILI, G., LISTA, F. & REZZA, G. The Italian 2017 Outbreak Chikungunya Virus Belongs to an Emerging Aedes albopictus–Adapted Virus Cluster Introduced From the Indian Subcontinent. Open forum infectious diseases, 2018. Oxford University Press US, ofy321.
43. LINTHICUM, K., DAVIES, F., KAIRO, A. & BAILEY, C. 1985. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Epidemiology & Infection,* 95**,** 197-209.
44. LUNDSTRÖM, J. O. & PFEFFER, M. 2010. Phylogeographic structure and evolutionary history of Sindbis virus. *Vector-Borne and Zoonotic Diseases,* 10**,** 889-907.
45. MACKENZIE, J. S. & JEGGO, M. 2013. Reservoirs and vectors of emerging viruses. *Current Opinion in Virology,* 3**,** 170-179.
46. MAVALANKAR, D., SHASTRI, P., BANDYOPADHYAY, T., PARMAR, J. & RAMANI, K. V. 2008. Increased mortality rate associated with chikungunya epidemic, Ahmedabad, India. *Emerging Infectious Diseases,* 14**,** 412.
47. MCINTOSH, B. 1970. Antibody against Chikungunya virus in wild primates in Southern Africa. *The South African Journal of Medical Sciences,* 35**,** 65.
48. MCINTOSH, B. 1972. Rift valley fever: 1. Vector studies in the field. *Journal of the South African Veterinary Association,* 43**,** 391-395.
49. MCINTOSH, B., JUPP, P., DOS SANTOS, I. & BARNARD, B. 1980. Vector studies on Rift Valley fever virus in South Africa. *South African Medical Journal,* 58**,** 127-32.
50. MCINTOSH, B., JUPP, P., DOS SANTOS, I. & MEENEHAN, G. 1976. Epidemics of West Nile and Sindbis viruses in South Africa with Culex (Culex) univittatus Theobald as vector. *South African Journal of Science,* 72**,** 295-300.

51. MCINTOSH, B., MCGILLIVRAY, G., DICKINSON, D. & MALHERBE, H. 1964. Illness caused by Sindbis and West Nile viruses in South Africa. *South African Medical Journal,* 38.
52. MCINTOSH, I. & JUPP, P. 1977. Rural epidemic of chikungunya in South Africa with involvement of Aedes (Diceromyia) furcifer (Edwards) and baboons. *South African Journal of Science,* 73**,** 267.
53. MORRILL, J., MEBUS, C. & PETERS, C. 1997. Safety of a mutagen-attenuated Rift Valley fever virus vaccine in fetal and neonatal bovids. *American Journal of Veterinary Research,* 58**,** 1110-1114.
54. MORRISON, J. 1979. Chikungunya fever. *International Journal of Dermatology,* 18**,** 628-629.
55. MOYEN, N., THIBERVILLE, S.-D., PASTORINO, B., NOUGAIREDE, A., THIRION, L., MOMBOULI, J.-V., DIMI, Y., LEPARC-GOFFART, I., CAPOBIANCHI, M. R. & LEPFOUNDZOU, A. D. 2014. First reported chikungunya fever outbreak in the republic of Congo, 2011. *PloS one,* 9**,** e115938.
56. MUSPRATT, J., SMITHBURN, K., PATERSON, H. & KOKERNOT, E. 1957. Studies on arthropod-borne viruses of Tongaland. X. The laboratory transmission of Wesselsbron virus by the bite of Aedes (Banksinella) circumluteolus Theo. *South African Journal of Medical Sciences,* 22**,** 121-126.
57. NARANG, P., DANGI, M., SHARMA, D., KHICHI, A. & CHHILLAR, A. K. 2019. An Integrated Chikungunya Virus Database to Facilitate Therapeutic Analysis: ChkVDb. *Current Bioinformatics,* 14**,** 323-332.
58. NJENGA, M. K., NDERITU, L., LEDERMANN, J., NDIRANGU, A., LOGUE, C., KELLY, C., SANG, R., SERGON, K., BREIMAN, R. & POWERS, A. 2008. Tracking epidemic chikungunya virus into the Indian Ocean from East Africa. *The Journal of general Virology,* 89**,** 2754.
59. OU, J.-H., STRAUSS, E. G. & STRAUSS, J. H. 1981. Comparative studies of the 3′-terminal sequences of several alphavirus RNAs. *Virology,* 109**,** 281-289.
60. OUILIBONA, R. C. S., SIMO, H. D. T., VICKOS, U., BERTHET, N. & NAKOUNÉ, E. 2018. Full-Length Genome Sequence of a Sindbis Virus Strain Isolated from Culex cinereus in 1977 in Bozo, Central African Republic. *Genome announcements,* 6.
61. PFEFFER, M. & DOBLER, G. 2010. Emergence of zoonotic arboviruses by animal trade and migration. *Parasites & Vectors,* 3**,** 35.
62. PICKERING, P., AASKOV, J. G. & LIU, W. 2019. Complete Genomic Sequence of an Australian Sindbis Virus Isolated 44 Years Ago Reveals Unique Indels in the E2 and nsP3 Proteins. *Microbiology Resource Announcements,* 8**,** e00246-19.
63. PIENAAR, N. J. & THOMPSON, P. N. 2013. Temporal and spatial history of Rift Valley fever in South Africa: 1950 to 2011. *Onderstepoort Journal of Veterinary Research,* 80**,** 1-13.
64. PYKE, A. T., MOORE, P. R. & MCMAHON, J. 2018. New insights into chikungunya virus emergence and spread from Southeast Asia. *Emerging Microbes & Infections,* 7**,** 26.
65. ROBIN, S., RAMFUL, D., LE SEACH, F., JAFFAR-BANDJEE, M.-C., RIGOU, G. & ALESSANDRI, J.-L. 2008. Neurologic manifestations of pediatric chikungunya infection. *Journal of Child Neurology,* 23**,** 1028-1035.
66. RÜCKERT, C., WEGER-LUCARELLI, J., GARCIA-LUNA, S. M., YOUNG, M. C., BYAS, A. D., MURRIETA, R. A., FAUVER, J. R. & EBEL, G. D. 2017. Impact of simultaneous exposure to arboviruses on infection and transmission by Aedes aegypti mosquitoes. *Nature Communications,* 8**,** 15412.
67. SALEH, S. M., POIDINGER, M., MACKENZIE, J. S., BROOM, A. K., LINDSAY, M. D. & HALL, R. A. 2003. Complete genomic sequence of the Australian south-west genotype of Sindbis virus: comparisons with other Sindbis strains and identification of a unique deletion in the 3′-untranslated region. *Virus Genes,* 26**,** 317-327.
68. SANE, J., KURKELA, S., PUTKURI, N., HUHTAMO, E., VAHERI, A. & VAPALAHTI, O. 2012. Complete coding sequence and molecular epidemiological analysis of Sindbis virus isolates from mosquitoes and humans, Finland. *Journal of General Virology,* 93**,** 1984-1990.

69. SCHEUCH, D., SCHÄFER, M., EIDEN, M., HEYM, E., ZIEGLER, U., WALTHER, D., SCHMIDT-CHANASIT, J., KELLER, M., GROSCHUP, M. & KAMPEN, H. 2018. Detection of Usutu, Sindbis, and Batai viruses in mosquitoes (Diptera: Culicidae) collected in Germany, 2011–2016. *Viruses,* 10**,** 389.
70. SCHUFFENECKER, I., ITEMAN, I., MICHAULT, A., MURRI, S., FRANGEUL, L., VANEY, M.-C., LAVENIR, R., PARDIGON, N., REYNES, J.-M. & PETTINELLI, F. 2006. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Medicine,* 3**,** e263.
71. SELLERS, R., PEDGLEY, D. & TUCKER, M. 1982. Rift Valley fever, Egypt 1977: disease spread by windborne insect vectors? *Veterinary Record,* 110**,** 73-77.
72. SHARP, B., LE SUEUR, D. & RIDL, F. 1988. Host preference studies on Aedes (Aedimorphus) durbanensis (Theobald). *Journal of the Entomological Society of Southern Africa*.
73. SIMPSON, D. A., DAVIS, N. L., LIN, S.-C., RUSSELL, D. & JOHNSTON, R. E. 1996. Complete nucleotide sequence and full-length cDNA clone of SA AR86, a South African alphavirus related to Sindbis. *Virology,* 222**,** 464-469.
74. STAPLEFORD, K. A., MORATORIO, G., HENNINGSSON, R., CHEN, R., MATHEUS, S., ENFISSI, A., WEISSGLAS-VOLKOV, D., ISAKOV, O., BLANC, H. & MOUNCE, B. C. 2016. Whole-genome sequencing analysis from the chikungunya virus Caribbean outbreak reveals novel evolutionary genomic elements. *PLoS Neglected Tropical Diseases,* 10**,** e0004402.
75. STORM, N., WEYER, J., MARKOTTER, W., LEMAN, P., KEMP, A., NEL, L. & PAWESKA, J. 2013. Phylogeny of Sindbis virus isolates from South Africa. *Southern African Journal of Epidemiology and Infection,* 28**,** 207-214.
76. STRAUSS, E. G., RICE, C. M. & STRAUSS, J. H. 1984. Complete nucleotide sequence of the genomic RNA of Sindbis virus. *Virology,* 133**,** 92-110.
77. SWANEPOEL, R., MANNING, B. & WATT, J. 1979. Fatal Rift Valley fever of man in Rhodesia. *Central African Journal of Medicine,* 25**,** 1-8.
78. TSETSARKIN, K. A., CHEN, R., SHERMAN, M. B. & WEAVER, S. C. 2011. Chikungunya virus: evolution and genetic determinants of emergence. *Current Opinion in Virology,* 1**,** 310-317.
79. TURELL, M., LINTHICUM, K. & BEAMAN, J. 1990. Transmission of Rift Valley fever virus by adult mosquitoes after ingestion of virus as larvae. *The American Journal of Tropical Medicine and Hygiene,* 43**,** 677-680.
80. UEJIO, C. K., KEMP, A. & COMRIE, A. C. 2012. Climatic controls on West Nile virus and Sindbis virus transmission and outbreaks in South Africa. *Vector-Borne and Zoonotic Diseases,* 12**,** 117-125.
81. VAN DEN BERGH, C., VENTER, E. H., SWANEPOEL, R. & THOMPSON, P. N. 2019. High seroconversion rate to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal, South Africa, in the absence of reported outbreaks. *PLOS Neglected Tropical Diseases,* 13**,** e0007296.
82. VENTER, M. 2018. Assessing the zoonotic potential of arboviruses of African origin. *Current Opinion in Virology,* 28**,** 74-84.
83. VOLK, S. M., CHEN, R., TSETSARKIN, K. A., ADAMS, A. P., GARCIA, T. I., SALL, A. A., NASAR, F., SCHUH, A. J., HOLMES, E. C. & HIGGS, S. 2010. Genome-scale phylogenetic analyses of chikungunya virus reveal independent emergences of recent epidemics and various evolutionary rates. *Journal of Virology,* 84**,** 6497-6504.
84. WANG, J., ZHANG, H., SUN, X., FU, S., WANG, H., FENG, Y., WANG, H., TANG, Q. & LIANG, G.-D. 2011. Distribution of mosquitoes and mosquito-borne arboviruses in Yunnan Province near the China–Myanmar–Laos border. *The American Journal of Tropical Medicine and Hygiene,* 84**,** 738-746.

85. WEAVER, S. C., KANG, W., SHIRAKO, Y., RUMENAPF, T., STRAUSS, E. G. & STRAUSS, J. H. 1997. Recombinational history and molecular evolution of western equine encephalomyelitis complex alphaviruses. *Journal of Virology,* 71**,** 613-623.
86. WEAVER, S. C. & REISEN, W. K. 2010. Present and future arboviral threats. *Antiviral Research,* 85**,** 328-345.
87. WEINBREN, M., KOKERNOT, R. & SMITHBURN, K. 1956. Strains of Sindbis-Like Virus isolated from Cu Heine Mosquitoes in the Union of South Africa. I. Isolation and Properties. *South African Medical Journal,* 30**,** 631-6.
88. WORTH, C.B., PATERSON, H.E. & DE MEILLON, B., 1961. The incidence of arthropod-borne viruses in a population of culicine mosquitoes in Tongaland, Union of South Africa (January, 1956, through April, 1960). The American Journal of Tropical Medicine and Hygiene, 10(4), pp.583-592.
89. ZHOU, G., LIANG, G., LI, L., FU, S., LI, F., JIN, Q., ZHANG, H., HUANG, W. & HOU, Y. 1999. The complete nucleotide sequence of the structural region gene of the Alphavirus YN87448 strain isolated in China and its relationship to other sindbis viruses. *Chinese Journal of Virology,* 15**,** 205-211.

Chapter 6

GENERAL CONCLUSION

Rift Valley fever is a re-emerging vector-borne zoonosis that reoccurs at long, irregular intervals in South Africa. Three large outbreaks have occurred the past 70 years in South Africa mainly following unusually high rainfall on the relatively dry central plateau. However, several smaller outbreaks have been recorded in both the interior and the eastern parts of the country during the IEPs (Pienaar and Thompson, 2013). The fate of the virus during the long IEPs in South Africa is poorly understood. Multiple reports have evidence of circulation of the virus during IEPs in the absence of clinical cases or vaccination, in livestock (Rostal *et al*., 2010, Sumaye *et al*., 2013, Fafetine *et al*., 2013), in wildlife (Britch *et al*., 2013, Fagbo *et al*., 2014) and in humans (Olaleye *et al*., 1996, LaBeaud, 2008). The wildlife-domestic animal interface, the presence of competent vectors in the game parks in northern KZN, the historical isolation of RVFV from mosquitoes in the area (Kokernot 1957), and serological and virological evidence from neighbouring Mozambique (Fafetine *et al*., 2013, Fafetine *et al*., 2016, Moiane *et al*., 2017) suggested that this low-lying, tropical area, the main study area for this study, may be an area of endemic RVFV circulation in South Africa.

The aim of the study was to investigate whether there was evidence of recent circulation of RVFV, and if so to estimate the seroprevalence in livestock and the rate of seroconversion. The observed seroprevalence was 34% in cattle and 32% in goats, with no evidence of any clinical signs of the disease in the study area. The seroconversion rate in cattle was 0.59 seroconversions per animal-year, while in goats it was 0.41 seroconversions per animal-year. The results indicate that RVFV is circulating in the area at a rate that varies by location, season and year. This is the first published study to quantify the seroconversion rate of RVF in livestock using the gold standard serum neutralization test (OIE, 2012). Previous studies illustrated inter epidemic circulation using ELISA-based detection of IgG and IgM antibodies. The seroprevalence during this study was high in all age groups. It is expected that a high seroprevalence among young animals might be a result of a recent outbreak. Since it is unlikely that a large outbreak will go unnoticed, the high seroprevalence in all age groups are more likely an indication of a hyperendemic

situation. Low level viral activity was present even during the dry winter months despite the drought conditions during the study. The seroprevalence in livestock for this area in 1955 was 12% (Kokernot *et al*., 1962), indicating an increase in the level of exposure to RVFV over the past 50 years. The livestock and human population for the same area has increased significantly resulting in an increase in RVFV host density. However there are no sheep in the area and intensive breeding is not practised. Animals are left to graze freely and can breed throughout the year. Together with the moderate herd immunity, this makes it unlikely that large outbreaks will occur or to make the recording of abortions more difficult.

To our knowledge there has never been a report of clinical RVF in humans in the area, and the last serological survey that was done in 1950, found that 16% (19/118) human sera had RVFV specific antibodies (Smithburn *et al*., 1959). However, the fact that malaria is common in this area means that other causes of febrile illness are likely to be overlooked. In support of this hypothesis, a recent study at a primary health care facility in Maputo, Mozambique, 100 km north of our study area, reported a 10% RVFV IgG seroprevalence in febrile patients (Gudo *et al*., 2016). Follow-up samples showed that 5% of patients seroconverted to RVFV, concluding that RVFV infections are not diagnosed or misdiagnosed as malaria, since laboratory diagnostics are not always done to confirm the disease (Gudo *et al*., 2016). This might also be true for other tropical parts of Africa. Therefore, the current status and potential impact of RVFV infection in humans in this study area, and in similar areas along the coastal plain of southern Africa and elsewhere, require further investigation.

Livestock tested in this study were from an area adjoining two nature reserves and were largely separated from wildlife by fences. In order to investigate the potential role of wildlife in RVFV circulation in the area, the seroprevalence and associated risk factors of RVFV in antelope in the Tembe Elephant Park and the Ndumo Game Reserve, using sera from animals routinely culled over a two-year period were investigated. The seroprevalence in nyala was 34%, and in impala 46%, in the absence of reported animal cases. Risk factors associated with seroprevalence to RVFV include the proximity to floodplain or swamps, and possibly due to larger skin

surface area. The role of different wildlife species in RVF epidemiology and the effects of the virus on their health require further investigation.

No recent studies have been published on the population dynamics of potential RVFV vectors in KZN and, with the results on the current RVFV circulation obtained in this study, it was evident that further investigation is required. Although limited in the area covered, this study was able to assess the current population diversity and dynamics of mosquitoes in the study area. Mosquito diversity differed between habitats regarding the water source available (standing vs flooding) and vegetation. Results indicated that cumulative rainfall and average minimum temperature over the 30 days before collection had an impact on the number of mosquitoes collected. The mosquito population composition appears to have changed over time, likely due to changes in abundance of different hosts, and that more extensive studies are warranted to better understand the population composition and dynamics of arbovirus vectors in the area. In this study it was found that Cx. (*Cux). tritaeniorhynchus*, was the most abundant species, followed by *Cx. (Cux.) antennatus.* Permanent water sources were available at all collection sites, favouring the breeding environment for *Culex* species. A limitation of this study was manpower and time. Since blood samples had to be collected at 5h00 in the morning, when herdsmen brought their animals to the dip tanks, the time to collect the mosquitoes was limited. This made it only possible to collect at two sites that was close to the dip tanks. In future more collection sites positioned further apart should be used. Location sites must also include different habitats that can vary from trees, open fields close to human settlements, different hosts e.g. gaze veld vs nature reserves. Mosquito collection methods, location and bait during collection also have an impact on the number and species collected. Another limitation during this time was that only one trap method e.g. collection nets were used and only one bait e.g. CO_2 in the form of dry ice, were used during collections. As the population dynamics in an area are driven by host preference, various live baits might be a consideration for future studies. Different collection methods for example light traps as well as different collection sites might be considered for future studies.

The mosquitoes collected during the study were screened for RVFV using RT-PCR, detecting RVFV RNA in a single pool of *Ae. (Adm.) durbanensis* collected in March 2017. Infection rate in vector populations are as low as 0.1% even during outbreaks (Swanepoel and Coetzer, 2004). The minimum rate infection for the study area in March 2017 was 0.33 per 1000 *Ae. (Adm.) durbanensis*, or 0.033%. In a previous study attempts were made to isolate RVFV from 340,000 mosquitoes collected in the same areas of northern KZN but were not successful (Worth *et al*., 1961; McIntosh *et al*., 1972). In our study, the single RVFV detection, along with the high seroprevalences observed in livestock and wildlife, allows us to conclude that the study area is a RVFV endemic area. Phylogenetic analysis of the RVFV sequence indicated that the virus was closely related to one obtained at Simbu pan, KZN in 1955 the same area, suggesting that the virus has been continuously circulating in the area for more than 60 years. It was also closely related to an isolate from the initial outbreak in South Africa in 1951, raising the possibility that the outbreak might have been due to animal movement from an endemic area to the interior of the county. The risk of moving animals from an endemic area such as KZN to the more vulnerable inner parts of South Africa must be investigated.

The nucleotide sequence of the RVFV obtained from the mosquito pool in this study was distinctly different from Lineage C viruses, which were responsible for outbreaks in Komatipoort, South Africa in 2008 (Pienaar and Thompson, 2013) and Maputo Province, Mozambique in 2014 (Fafetine *et al*., 2016) fairly close to our study area. Further investigation should be done to confirm the possibility that there may be multiple lineages co-circulating in the area, as reported in Kenya and Zimbabwe (Swanepoel *et al*., 1979, Ikegami, 2012).

Several of the most abundant mosquito species identified in this study have been implicated as vectors elsewhere; *Cx. tritaeniorhynchus,* has been identified as a vector in Saudi Arabia and Yemen in 2000 (Miller *et al*., 2002), *Cx. antennatus*, a vector in Egypt (Gad *et al*., 1995); *Cx. neavei*, in South Africa in 1981 (McIntosh and Jupp, 1981); *Cx. neavei*, in Mauritania in 2003 (Faye *et al*., 2007); *Ae. circumluteolus*, from which RVFV was isolated in this study area in 1955 (Kokernot *et al*., 1957)*; Ae. mcintoshi*, in which transovarial transmission was demonstrated in Kenya (Linthicum

et al., 1985); *Ae. durbanensis,* implicated in causing RVF disease symptoms in sheep in Kenya in 1937(Steyn and Schulz, 1955). Since Chikungunya and Sindbis viruses were also detected in the same *Ae. durbanensis* mosquito pool, further investigation should be aimed to establish whether *Ae. durbanensis* is a possible competent vector for RVFV, Chikungunya and Sindbis viruses. It is also the first time that RVF Chikungunya or Sindbis virus have been isolated from this mosquito species.

The Sindbis virus was related to the Girdwood strain that was isolated in South Africa in 1996. The Chikungunya virus was closely related to two isolates from an outbreak in South Africa in 1976; both of these obtained several hundred km away from the study area, raising the question “of how did they get there”? The increasing human population in the study area has increased the potential host density, and increased long distance movement of people may have play a role. It is possible that Chikungunya and Sindbis viruses are circulating with RVFV in the area and are possibly misdiagnosed in humans since the symptoms of these virus infections are fever and non-specific in many cases.

The results of this study conclusively show the presence of RVFV in the area. The most likely explanation for the similar seroprevalence in both wildlife and domestic ruminants, along with the presence of RVFV among vectors is that they are all part of the same vector-host maintenance system. The definition of a reservoir depends on specifying the target population (Haydon *et al*., 2002); therefore, it is possible that the wildlife-vector system may act as a reservoir for livestock, or the livestock-vector system may act as a reservoir for wild ruminants. In terms of the potential health risk to humans, all three components (livestock, wildlife and vector) may constitute the reservoir. More research should be done on the mechanisms of maintenance of viral circulation of the virus in the environment. Questions that need more investigation are: could the low-level circulation be maintained by only horizontal transmission (host – vector-host) or does it require vertical transmission (transovarial transmission in the vector) or does a combination occur. In a study in the Kruger National Park in African buffaloes it was concluded that a combination of horizontal and vertical transmission by mosquitoes was necessary for RVFV maintenance (Manore and Beechler, 2015).

Further investigation should be done into the factors affecting viral circulation and survival, and the impact of the presence of RVFV on animal and human health.

REFERENCES:

1. BRITCH, S. C., BINEPAL, Y. S., RUDER, M. G., KARIITHI, H. M., LINTHICUM, K. J., ANYAMBA, A., SMALL, J. L., TUCKER, C. J., ATEYA, L. O. & ORIKO, A. A. 2013. Rift Valley fever risk map model and seroprevalence in selected wild ungulates and camels from Kenya. *PLoS One,* 8**,** e66626.
2. FAFETINE, J., NEVES, L., THOMPSON, P. N., PAWESKA, J. T., RUTTEN, V. P. & COETZER, J. A. 2013. Serological evidence of Rift Valley fever virus circulation in sheep and goats in Zambezia Province, Mozambique. *PLoS Neglected Tropical Diseases,* 7**,** e2065.
3. FAFETINE, J. M., COETZEE, P., MUBEMBA, B., NHAMBIRRE, O., NEVES, L., COETZER, J. A. & VENTER, E. H. 2016. Rift Valley fever outbreak in livestock, Mozambique, 2014. *Emerging Infectious Diseases,* 22**,** 2165.
4. FAGBO, S., COETZER, J. A. & VENTER, E. H. 2014. Seroprevalence of Rift Valley fever and lumpy skin disease in African buffalo (Syncerus caffer) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa. *Journal of the South African Veterinary Association,* 85**,** 01-07.
5. FAYE, O., DIALLO, M., DIOP, D., BEZEID, O. E., BÂ, H., NIANG, M., DIA, I., MOHAMED, S. A. O., NDIAYE, K. & DIALLO, D. 2007. Rift Valley fever outbreak with East-Central African virus lineage in Mauritania, 2003. *Emerging infectious diseases,* 13**,** 1016.
6. GAD, A. M., RIAD, I. B. & FARID, H. A. 1995. Host-feeding patterns of Culex pipiens and Cx. antennatus (Diptera: Culicidae) from a village in Sharqiya Governorate, Egypt. *Journal of medical entomology,* 32**,** 573-577.
7. GUDO, E. S., PINTO, G., WEYER, J., LE ROUX, C., MANDLAZE, A., JOSÉ, A. F. & PAWESKA, J. T. 2016. Serological evidence of Rift Valley fever virus among acute febrile patients in Southern Mozambique during and after the 2013 heavy rainfall and flooding: implication for the management of febrile illness. *Virology journal,* 13**,** 96.
8. IKEGAMI, T. 2012. Molecular biology and genetic diversity of Rift Valley fever virus. *Antiviral Research,* 95**,** 293-310.
9. JUPP, P. & MCINTOSH, B. 1987. A bionomic study of adult Aedes (Neomelaniconion) circumluteolus in northern Kwazulu, South Africa. *Journal of the American Mosquito Control Association,* 3**,** 131-136.
10. KOKERNOT, R., HEYMANN, C., MUSPRATT, J. & WOLSTENHOLME, B. 1957. Studies on arthropod-borne viruses of Tongaland. V. Isolation of Bunyamwera and Rift Valley Fever viruses from mosquitoes. *South African Journal of Medical Sciences,* 22**,** 71-80.
11. KOKERNOT, R., MCINTOSH, B., WORTH, C. B., DE MORAIS, T. & WEINBREN, M. 1962. Isolation of Viruses from Mosquitoes Collected at Lumbo, Mozambique. *The American journal of tropical medicine and hygiene,* 11**,** 678-682.
12. LABEAUD, A. D. 2008. Why arboviruses can be neglected tropical diseases. *PLoS Neglected Tropical Diseases,* 2**,** e247.
13. LINTHICUM, K., DAVIES, F., KAIRO, A. & BAILEY, C. 1985. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Epidemiology & Infection,* 95**,** 197-209.
14. MCINTOSH, B. 1972. Rift valley fever: 1. Vector studies in the field. *Journal of the South African Veterinary Association,* 43**,** 391-395.
15. MCINTOSH, A., JUPP, P. & DOS SANTOS, I. 1983. Field and laboratory evidence implicating Culex zombaensis and Aedes circumluteolus as vectors of Rift Valley fever virus in coastal South Africa. *South African Journal of Science,* 79**,** 61-64.
16. MCINTOSH, B. & JUPP, P. Epidemiological aspects of Rift Valley fever in South Africa with reference to vectors. Proceedings... Rift Valley fever; a workshop, 1981.

17. MILLER, B. R., GODSEY, M. S., CRABTREE, M. B., SAVAGE, H. M., AL-MAZRAO, Y., AL-JEFFRI, M. H., ABDOON, A.-M. M., AL-SEGHAYER, S. M., AL-SHAHRANI, A. M. & KSIAZEK, T. G. 2002. Isolation and genetic characterization of Rift Valley fever virus from Aedes vexans arabiensis, Kingdom of Saudi Arabia. *Emerging infectious diseases,* 8**,** 1492.
18. MOIANE, B., MAPACO, L., THOMPSON, P., BERG, M., ALBIHN, A. & FAFETINE, J. 2017. High seroprevalence of Rift Valley fever phlebovirus in domestic ruminants and African buffaloes in Mozambique shows need for intensified surveillance. *Infection ecology & epidemiology,* 7**,** 1416248.
19. OIE 2012. World Organisation for Animal Health. Rift Valley fever. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (7th ed.). Paris.
20. OLALEYE, O., TOMORI, O. & SCHMITZ, H. 1996. Rift Valley fever in Nigeria: infections in domestic animals. *Revue scientifique et technique-Office international des épizooties,* 15**,** 937-946.
21. PIENAAR, N. J. & THOMPSON, P. N. 2013. Temporal and spatial history of Rift Valley fever in South Africa: 1950 to 2011. *Onderstepoort Journal of Veterinary Research,* 80**,** 1-13.
22. ROSTAL, M. K., EVANS, A. L., SANG, R., GIKUNDI, S., WAKHULE, L., MUNYUA, P., MACHARIA, J., FEIKIN, D. R., BREIMAN, R. F. & NJENGA, M. K. 2010. Identification of potential vectors of and detection of antibodies against Rift Valley fever virus in livestock during interepizootic periods. *American Journal of Veterinary Research,* 71**,** 522-526.
23. SMITHBURN, K., HEYMANN, C., WEINBREIN, M. & ZENTKOWSKY, D. 1959. Neutralizing antibodies for certain viruses in the sera of human beings residing in northern Natal. *South African Medical Journal,* 33.
24. STEYN, J. & SCHULZ, K. 1955. Aëdes (Ochlerotatus) caballus Theobald, the South African vector of Rift Valley fever. *South African Medical Journal,* 29**,** 1114-20.
25. SUMAYE, R. D., GEUBBELS, E., MBEYELA, E. & BERKVENS, D. 2013. Inter-epidemic transmission of Rift Valley fever in livestock in the Kilombero River Valley, Tanzania: a cross-sectional survey. *PLoS Neglected Tropical Diseases,* 7**,** e2356.
26. SWANEPOEL, R. & COETZER, J. 2004. Rift valley fever. *Infectious Diseases of Livestock,* 2**,** 1037-1070.
27. SWANEPOEL, R., MANNING, B. & WATT, J. 1979. Fatal Rift Valley fever of man in Rhodesia. *Central African Journal of Medicine,* 25**,** 1-8.
28. WORTH, C.B., PATERSON, H.E. & DE MEILLON, B., 1961. The incidence of arthropod-borne viruses in a population of culicine mosquitoes in Tongaland, Union of South Africa (January, 1956, through April, 1960). *The American Journal of Tropical Medicine and Hygiene*, 10(4), pp.583-592.

APPENDIX

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

ANIMAL ETHICS COMMITTEE
Private Bag X04
0110 Onderstepoort

Tel +27 12 529 8434 / Fax +27 12 529 8300
e-mail: aec@up.ac.za

Ref: V013-16

19 April 2016

Dear Prof Venter

Department of Veterinary Tropical Diseases

Faculty of Veterinary Sciences

Epidemiological dynamics of RVFV in S.A. Serological surveys to detect exposure to RVF in various parts of S.A. (C v.d Bergh)

The above application was approved by the UP-Animal Animal Ethics Committee held on 18 April 2016

Kind regards

Prof Vinny Naidoo

Deputy Dean: Research and Postgraduate Studies

Director: Biomedical Research Centre

Faculty of Veterinary Science

University of Pretoria

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

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

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Carien van den Bergh
Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
Onderstepoort
Tel: 082 300 7406
E-mail: cvdbprivate@fastmail.fm

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Dear Carien van den Bergh

Your application sent with the email on 4 May 2016 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 study, "*Epidemiological dynamics of Rift Valley fever in southern Africa*" 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. The study must be conducted in compliance with the Veterinary and Para-Veterinary Professions Act 1982 (Act No. 19 of 82);
3. Prior consent from the owners of animals to be used in the study must be obtained in writing;

4. All samples must be packaged and transported in compliance with the National Road Traffic Act 1996 (Act no 93 of 1996);
5. All samples must travel under the cover of a Red Cross Permit issued by the state veterinarian of the area of origin and in full compliance with all the conditions included on such a permit;
6. All samples must be transported without deviation to the BSL 2+ facility at the Department of Veterinary Tropical Diseases;
7. Serum and EDTA samples must be inactivated at 56°C for 1 hour before any other handling or processing of the samples may take place;
8. Inactivation of serum and EDTA samples must be performed under the control of the person responsible for the laboratory. This person must issue written confirmation of the sample identification numbers, temperature and time at which the heat inactivation was conducted;
9. All testing and extractions must be performed in the BSL 2+ facility at the Department of Veterinary Tropical Diseases;
10. No virus isolation may be performed on any sample of any origin unless written permission has been obtained from the Director Animal Health. Application must be made in writing to HerryG@daff.gov.za;
11. As Rift Valley Fever is a notifiable disease in terms of the Animal Diseases Act 1984 (Act no 35 of 84), all results of testing must be copied to the state veterinarian of the area. Results may not be distributed;
12. All potentially infectious material utilised or generated during or by the study is to be destroyed at completion of the study by incineration;
13. Only a registered waste disposal company may be used for the removal of waste generated during the study;
14. Any suspicion of a controlled or notifiable disease in terms of the Animal Disease Act 1984 (Act no 35 of 84) must immediately be reported to the local state veterinarian;
15. Records must be kept for five years for audit purposes;
16. A dispensation for the storage of extracted RNA and left over blood samples is attached.

Please note that there is no properly validated serological test for Rift valley Fever at the moment in South Africa. We would thus like to urge caution in interpreting results for Rift Valley Fever in this study

Title of research/study: "*Epidemiological dynamics of Rift Valley fever in southern Africa*"
Researcher: Carien van den Bergh
Institution: BSL 2+ Department of Veterinary Tropical Diseases;
Our ref Number: 12/11/1/1/6

Kind regards,

MMaja.

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH

Date: 2016 -05- 13

SUBJECT: *PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)*

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

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

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Carien van den Bergh
Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
Onderstepoort
Tel: 082 300 7406
E-mail: cvdbprivate@fastmail.fm

RE: DISPENSATION ON SECTION 20 APPROVAL IN TERMS OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) FOR: "EPIDEMIOLOGICAL DYNAMICS OF RIFT VALLEY FEVER IN SOUTHERN AFRICA"

A dispensation is hereby granted on Point 11 of the Section 20 approval that was issued for the above mentioned study (attached):

i) Left over blood samples that have been inactivated at 56°C for 1 hour must be stored under access control in the BSL 2+ facility at the at the Department of Veterinary Tropical Diseases;

ii) Extracted RNA must be stored under access control in the BSL 2+ facility at the at the Department of Veterinary Tropical Diseases

iii) Stored samples may not be outsourced or used for further research without prior written approval from DAFF.

Kind regards,

DR. MPHO MAJA
DIRECTOR: ANIMAL HEALTH
Date: 2016 -05- 13