Retrospective study on antibody response to vaccination of the African buffalo (*Syncerus caffer*) and roan antelope (*Hippotragus equinus*) with Clone 13 Rift Valley fever virus vaccine

by

Peter Stanley Brothers

Submitted in partial fulfilment of the requirements for the degree

Master of Veterinary Medicine (Fer)

in the Department of Production Animal Studies,
Faculty of Veterinary Science,
University of Pretoria

Supervisor: Prof EH Venter (Department of Veterinary Tropical Diseases)
Co-Supervisors: Prof PT Thompson and Dr REJ Burroughs

June 2017

DECLARATION

I hereby declare that this dissertation, which I hereby submit for the MMedVet (Fer) degree in the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, to be my own work and has not been previously submitted by me for degree purposes at another tertiary institution.

_____

Dr PS Brothers BVSc, MSc (VTD)

February 2017

DEDICATION

To Becky, William and Olivia for the many hours of family time they sacrificed in order to make this possible – thank you, this is for you.

ACKNOWLEDGEMENTS

- Prof Estelle Venter, Dr Richard Burroughs, Prof Peter Thompson – for your guidance and patience

- East Cape Parks and Tourism Agency – Mr Brad Fike in particular

- Mr Neill Dodds – Nukamma Wildlife

- Dr William Fowlds

- IDEXX Laboratories South Africa – Johann Muller and Prof Fred Reyers in particular

- Dr Beate von Teichman – OBP – for your assistance and sound advice

- The Department of Veterinary Tropical Disease (DVTD), and specifically Ms Karen Ebersohn at the Faculty of Veterinary Science, Onderstepoort, University

- Funding of laboratory testing - E H Venter, DVTD, P Thompson PAS

- Brothers Safaris – funding of veterinary immobilisations and sample collection

SUMMARY

RETROSPECTIVE STUDY ON ANTIBODY RESPONSE TO VACCINATION OF THE AFRICAN BUFFALO (*SYNCERUS CAFFER*) AND ROAN ANTELOPE (*HIPPOTRAGUS EQUINUS*) WITH CLONE 13 RIFT VALLEY FEVER VIRUS VACCINE

By

Dr Peter Stanley Brothers

Supervisor: Prof EH Venter
Co-supervisors: Prof PN Thompson and Dr R Burroughs
Department: Production Animal Studies
Degree: MedVet (Fer)

Rift Valley fever (RVF) is a disease of ruminants in Africa, Madagascar and the Middle East, affecting primarily domestic, but also many wild species. The disease can be peracute to acute and is characterised by a necrotic hepatitis and a generalised haemorrhagic syndrome. The disease is caused by a mosquito-borne virus of the *Phenuiviridae* family. Outbreaks occur after heavy rains, which favour the breeding of the mosquito vectors. The disease is a zoonosis and humans become infected by bites from infected mosquitoes or contact with tissue from infected animals. The first records of RVF in southern Africa date back to 1950 when a large epidemic occurred in South Africa and there have been many outbreaks since, some major, with the most recent major outbreak in SA in 2010.

During the 2010 outbreak multiple indigenous wildlife species were affected, including springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus pygargus phillipsi*), bontebok (*D. pygargus pygargus*), waterbuck (*Kobus ellipsiprymnus*), African buffalo (*Syncerus caffer*), sable (*Hippotragus niger*), kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angasii*) and

gemsbok (*Oryx gazella*). Even though no cases have been recorded in roan (*Hippotragus equinus*) antelope to date, the fact that such a wide array of wildlife was affected, and taking into account the close phylogenetic relationship between sable and roan antelope, it is reasonable to assume that roan will also be susceptible to RVF.

Many control methods are aimed at vector control, but since the epidemiology of the disease is still poorly understood, this has limited value. Vaccination thus provides the best means of disease prevention and RVFV Clone 13 vaccine is a new vaccine proven to be effective and safe in domestic animals. It has not yet been scientifically tested in wildlife.

To date there is no published research on the use of RVF vaccine in wildlife, nor are there any recommended vaccine protocols for wildlife. Many of these species are valuable in conservation and financial terms, and hence the need for this research.

The purpose of the research was:

To determine whether Clone 13 RVFV vaccine is effective in creating a humoral immune response in the African savannah buffalo and roan antelope.
To determine if there are any obvious clinical side effects, such as general malaise or abortion due to vaccination.

The World Organisation for Animal Health (OIE) lists both the enzyme linked immunosorbent assay (ELISA) and serum neutralization test (SNT) as recommended methods for detecting an immune response in populations considered free from disease, as well as post-vaccination to detect an immune response. As reported previously, the ELISA and SNT have been used to detect antibodies against RVFV in serum of a variety of animal species.

Twenty-four buffalo and nine roan antelope were immobilised using standard immobilisation and capture techniques and all were bled for routine haematology and biochemistry screening as a general health screen. Both ELISA and SNT were applied on initial samples from all animals. All study subjects were vaccinated at first immobilisation and sample collection, following standard methodology. Thereafter subsequent blood samples were obtained at irregular intervals to test for the presence of antibodies specific to RVFV as a response to

vaccination. Due to their value and since no permission from owners was obtained to dart them at any stage for sample collection, sample collection mostly coincided with planned immobilisations for unrelated procedures on these animals.

All animals appeared in good health based on laboratory results and observation. Most of the buffalo showed a vaccination response, although this varied and did not last for a long period with titres dropping sooner than expected. With the exception of one individual, roan antelope showed a much better and longer lasting response to vaccination.

It is evident that RVF Clone 13 vaccination in buffalo cannot be considered as reliable protection in buffalo against disease, and that further work in this regard should be undertaken. This poor response can be due to a number of factors, but this is beyond the scope of this study. The roan antelope showed a better response in titre levels and duration. However, the low number of study subjects, and one individual exhibiting no response, precludes any reliable conclusions on vaccination response. These animals were also not exposed to disease challenge and thus efficacy in protection against disease could not be determined.

No animals showed any obvious side effects to vaccination, even those which were heavily pregnant at the time of vaccination. Although Clone 13 RVF vaccine does elicit a humoral immune response to RVF vaccination in the African savannah buffalo and roan antelope, it can thus not be relied upon to give adequate protection against RVF in these two species without further investigation.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1: INTRODUCTION

Rift Valley fever (RVF) is a disease primarily of domestic ruminants in Africa, Madagascar and the Middle East, which can be peracute to acute and is characterised by a necrotic hepatitis and a generalised haemorrhagic syndrome. There is considerable variation in the susceptibility to RVF of animals of different species and the disease is most severe in sheep, goats and cattle. Sudden mortality, neonatal mortality and abortion are common clinical signs, but infrequently inapparent or mild infections can occur in domestic animals (Swanepoel and Coetzer, 2004). The disease is caused by Rift Valley fever *Phlebovirus* (RVFV), a mosquito-borne virus in the genus *Phlebovirus* of the *Phenuiviridae* family. Outbreaks occur generally after very heavy rains which favour the breeding of the mosquito vectors (Swanepoel and Coetzer, 2004). Differential diagnoses include bluetongue, Nairobi sheep disease, heartwater, ephemeral fever, foot and mouth disease, leptospirosis and brucellosis, many of which also cause abortion (Davies and Martin, 2006).

The disease is a zoonosis and humans become infected most frequently by contact with tissue from infected animals (Van Velden, *et al*., 1977). Symptoms vary from mild influenza-like symptoms to more severe ocular disease, encephalitis and haemorrhagic fever associated with necrotic hepatitis and death (Freed, 1951; Gear, 1977; Swanepoel, *et al*., 1979; McIntosh, *et al*., 1980). In 2008, a South African outbreak in humans occurred predominantly in high risk groups, such as farmers and veterinarians (Archer, *et al*., 2011).

The disease was first recognised, and the virus isolated, in Kenya, with major outbreaks occurring in 1930-31, 1968 and 1978-79, and lesser outbreaks occurring in the years in-between (Stordy, 1913; Daubney, *et al*., 1931; Davies, *et al*., 1985; Meegan and Bailey, 1989). The first records of RVF in southern Africa date back to 1950 when a large epidemic occurred in South Africa (western Orange Free State, southern Transvaal (Gauteng Province) and bordering northern area of the Cape Province), although it was only recognized as RVF in 1951 after humans became ill following a necropsy of an infected animal (Alexander, 1951; Mundel and Gear, 1951). Since then there have been many minor and major outbreaks in South Africa, the most recent major outbreak being in 2010 (Pienaar and Thompson, 2013).

A low prevalence of RVF antibody was detected in antelope sera as far back as the early 1970's (Davies, 1975; Davies and Karstad, 1981) and in South Africa during the 1950-51 outbreak, abortions were noticed in some antelope species, although these could not be confirmed as a result of RVF (Joubert, Ferguson, Gear, 1951). More recently in the RVF epidemic of 2010 in South Africa multiple indigenous and exotic wildlife species were affected by RVF, including the African buffalo (Directorate of Animal Health 2012).

While a presumptive diagnosis can be made based on clinical signs (abortions in sheep, cattle and goats) and fatal disease, more specific diagnosis is required for a disease of this severity. Various laboratory tests such as serological and molecular assays can be used, or traditionally virus isolation and/or histopathology for confirmatory diagnosis (OIE, 2009).

Control of RVF is achieved either through vector control, limiting exposure to infected vectors or via immunization. Due to limitations with practical vector control and limiting exposure to infected vectors, especially in wildlife, immunization remains the only effective way to protect animals from RVFV infection. Several vaccines exist – the Smithburn live attenuated virus vaccine, modified live Smithburn vaccine, formalin-inactivated RVFV vaccines, Clone 13 live attenuated virus vaccine and a MP-12 live attenuated virus vaccine which is not available commercially.

While there has been much research on the use of the various vaccines in domestic animals, to date there is no published research regarding the use of RVF vaccines in wildlife. There are also no scientifically recommended vaccine protocols for wildlife.

This study aimed to determine whether Clone 13 RVFV vaccine is effective in creating a humoral immune response to RVFV in the African savannah buffalo (*Syncerus caffer*) and roan antelope (*Hippotragus equinus*).

CHAPTER 2: LITERATURE REVIEW

2.1 Aetiology

Rift Valley fever phlebovirus (RVFV) is a member of the Phlebovirus genus of the family *Phenuiviridae*, and exhibits typical morphological and physicochemical properties of this family and genus (Rice, *et al*., 1980; Ellis, *et al*., 1988; Sherman, *et al*., 2009). The virus structure is spherical in shape, with a lipid bilayer envelope and surface protrusions believed to be composed of two viral transmembrane glycoproteins (Freiberg, *et al*., 2008). RVFV has a tripartite, single stranded, negative-sense RNA genome. The large (L) RNA segment encodes for the L-protein which is the RNA-dependent RNA polymerase, while the medium (M) RNA segment encodes a polyprotein that cleaves into at least four proteins during translation (Gerrard, *et al*, 2007; Ikegami, *et al.*, 2007). These are: Gn glycoprotein; Gc glycoprotein and two non-structural proteins: NSm1 and NSm2 (Collett, 1986; Kakach, *et al.*, 1988). The non-structural protein 1 (NSm1) is a fusion protein containing the entire NSm and Gn sequences (Kakach, *et al*., 1988). The Gn and Gc proteins form the glycoprotein shell around the capsid (Schmaljohn and Nichol, 2007). The small (S) segment uses an ambisense strategy to encode the nucleoprotein N and non-structural protein NSs (Sherman, *et al*., 2009). Each of the three RNA segments binds N and L proteins which form ribonucleoprotein (RNP) complexes contained within the interior of the virus particle (Sherman, *et al l*., 2009). The glycoproteins are responsible for recognising receptor sites on susceptible cells, manifesting viral haemaglutinating ability and inducing a protective immune response (Besselaar and Blackburn, 1991). While no significant antigenic differences have been exhibited between RVFV isolates and laboratory-passaged strains, differences in pathogenicity have been demonstrated (Peters and Anderson, 1981; Anderson and Peters, 1988; Meegan and Bailey, 1989).

The virus is very stable in serum and can be recovered after several months of storage at 4°C, or after three hours at 56°C (Shimshony and Barzilai, 1983). RVFV is also very stable after freeze drying or at temperatures below minus 60°C, as well as in aerosols at 23°C and 50-85% humidity. The virus is inactivated by lipid solvents such as ether and sodium deoxycholate and low concentrations of formalin. Infection is lost rapidly if the pH drops below 6.8 (Shimshony and Barzilai, 1983).

2.2 Pathogenesis

RVFV infection shows unique pathogenesis in each animal model. Because viral replication and host antiviral responses most probably contribute to viral pathogenecity, an understanding of RVF pathogenesis requires identification and characterization of the viral virulence factors and host antiviral factors (Ikegami and Makino, 2011). Lambs are very susceptible to the disease and thus serve as a good model for considering the pathogenesis.

After RVFV attaches to receptors of susceptible cells these cells take up the virus by endocytosis, after which the virus replicates in the cytoplasm. Virions mature primarily by budding through the endoplasmic reticulum in the Golgi region into cytoplasmic vesicles. These vesicles are presumed to fuse with the plasma membrane, releasing virus, or virus can bud directly from the plasma membrane (Anderson and Smith, 1987).

RVFV infection causes an acute and fatal disease in newborn lambs and the mortality is 95 to 100% (Easterday, 1965). Newborn lambs infected with RVFV usually exhibit obvious illness, including elevated body temperature (40-41°C), loss of appetite, decreased activity, and prostration, about 12-18 h prior to death (Easterday, *et al*., 1962). Experimental infection studies in 1-4 day-old lambs with RVFV via the subcutaneous route resulted in necrosis of isolated hepatocytes (12-18 h post infection), focal coagulative necrosis of hepatocytes (24-33 h post infection), and extensive hepatocyte necrosis (48-51 h post infection) with a progressive increase in viral antigens, whereas no viral antigens could be detected in the endothelial or Kupffer cells in the liver. This suggests that the hepatocytes are the primary target of RVFV (Van der Lugt, *et al*., 1996). The necrosis is predominantly centrilobular or midzonal, and yet there is no definite distribution pattern in liver necrosis (Easterday, 1965; Coetzer, 1977). Some of the infected lambs also exhibited necrosis in the villi at the distal jejunum and ileum and depletion of lymphocytes in the spleen. The brain and eyes exhibited no lesions (Coetzer, 1977).

Generally, the liver pathology of newborn lambs resembles that of mice or hamsters, which are extremely susceptible to RVFV. However, the neurovirulence caused by this virus in lambs is less prominent when compared with that in rodents (Ikegami and Makino, 2011).

In comparison, 7-11-month-old Yansaka sheep inoculated subcutaneously with RVFV died during the viraemic febrile phase and displayed symptoms of epistaxis (two days post infection), severe and bloody diarrhoea, conjunctival haemorrhage, widespread petechiae and ecchymoses in hairless areas, pulmonary oedema/haemorrhage, and thrombi formation in the blood vessels of the heart, kidneys and brain. West African Dwarf sheep infected with RVFV did not exhibit such rapid haemorrhagic symptoms and rather exhibited marked coagulative hepatic necrosis and brain lesions, including mild gliosis, neural degeneration, neurophagia, and satellitosis (Olaleye, *et al*., 1996). This highlights the unique pathogenesis in each animal model, even within one species.

2.3 Epidemiology

2.3.1 Occurrence

From 1950 to 2011, one or more RVF outbreaks were reported in 27 seasons in South Africa. This included three major epidemics that affected an extensive area of the country (1950 – 1951, 1974–1976 and 2010–2011) (Weiss, 1957; Barnard and Botha, 1977; Coetzer, 1977; Pienaar and Thompson, 2013). The other outbreaks were smaller epidemics, individual outbreaks or isolated cases, some of which were not laboratory confirmed (Van der Linde, 1953; Weiss, 1957; McIntosh, 1972; Barnard and Botha, 1977; McIntosh, 1980; Pienaar and Thompson, 2013).

A small epidemic of RVF in South Africa occurred in 2008, affecting livestock (sheep, cattle and goats) as well as wildlife species and humans. The outbreak occurred in the four provinces of Mpumalanga, Limpopo, Gauteng and the North-West Province, while in 2009 smaller outbreaks of RVF were recorded in KwaZulu-Natal province. The 2008 and 2009 outbreaks were limited to certain areas of South Africa. In 2010 another major epidemic occurred (OIE, 2010; Directorate of Animal Health 2012), affecting the Free State province, areas of the Northern Cape, Eastern Cape, Gauteng, and Mpumalanga provinces and the Western Cape province. Again, both domestic livestock (sheep, cattle and goats) as well as different wildlife species were affected (OIE 2010). There we also many human cases, including fatalities.

Other southern African countries have also experienced outbreaks of disease over the years, including Zimbabwe (Swanepoel, 1976; Swanepoel, 1981), Mozambique (Petisca and Serra, 1971; McIntosh, 1972; Fafetine, *et al*., 2016) and Zambia (Hussein, *et al*., 1987; Dautu, *et al.*,

2012). Disease outbreaks, periodic virus isolation or serological evidence of RVFV have also been recorded in other African countries: in Madagascar (Meegan and Bailey, 1989), and more recently in Somalia, Kenya and Tanzania in 2006 and 2007 (WHO, 2007; Himeidan *et al*., 2014), the Comores in 2007 and 2008 (Sissoko, *et al.,* 2009), Mauritania in 2010 (El Mamy, *et al*., 2011) and again in 2012 (Sow *et al*., 2014), and Niger in 2016 (Tambo *et al.,* 2016).

Outside of Africa the first outbreaks of RVF were reported in Saudi Arabia and Yemen (Jupp, *et al*., 2002; Shoemaker, *et al*., 2002; Balkhy and Memish, 2003).

2.3.2 Inter-epidemic periods and vectors

Due to the fact that the mosquito is the primary vector for the virus, major outbreaks are usually associated with above average rainfall, which occurs at irregular intervals (Davies, *et al*., 1985). A major unknown factor in the epidemiology of the disease has been the question of where the virus is harboured during inter-epidemic periods. For decades, it was believed that it was endemic in indigenous forests, circulating between mosquitoes and unknown vertebrates, only spreading to livestock in (surrounding) areas when high rainfall allowed for the breeding of the mosquito vectors in epidemic proportions (Smithburn, *et al*., 1948; Smithburn, *et al*., 1949). This theory was reinforced by the finding of neutralising antibody to RVFV in cattle sera from herds grazing near the Knysna Forest (southern South Africa) in 1952, after the 1950-51 epidemic in South Africa (Du Toit, 1955). These cattle were nowhere near the outbreak of 1950-51. Similarly, neutralising antibody to RVFV was also discovered in human and livestock sera in the forested Tongaland area of northern KwaZulu-Natal after the outbreak, also nowhere near the affected areas. Here the virus was also isolated from aedine mosquitoes (Kaschula, 1953; Kokernot, *et al*., 1956; Kokernot, *et al*., 1957; Kokernot, *et al*., 1961).

It was not evident how close livestock had to be to forested areas for an outbreak to occur, nor how the virus seemed to travel hundreds of kilometres across mountain ranges, as mentioned in the examples above. One theory to explain this was that migratory birds carried/spread the infection, but to date no antibody has been demonstrated in birds, and they appear to be refractory to infection (Weiss, 1957; Davies and Addy, 1979).

The first mosquitoes from which the virus was isolated in southern Africa were *Aedes caballus sensu lato* and *Culex theileri*, collected at a pan in the western Free State during the 1953

outbreak in South Africa (Gear, *et al*., 1955). The virus has now been isolated from 12 species in the subcontinent: five *Aedes* spp., three *Culex* spp., three *Anopheles* spp., and one *Eretmapodites* spp. (Swanepoel and Coetzer, 2004).

During inter-epidemic periods (1982 and 1984) in Kenya, RVFV was isolated from unfed male and female Ae. mcintoshi mosquitoes (Linthicum, *et al*., 1985). This was a major breakthrough in understanding the epidemiology of RVF since it supported the theory that the virus was still present between epidemics, and that transovarial transmission was possible in Aedes mosquitoes. This finding also suggests that venereal transmission could occur from transovarially infected male mosquitoes to females (Linthicum, *et al*., 1985). It should be noted that inter-epidemic transmission of RVFV in cattle in Kenya was demonstrated by Scott, Weddell and Reid (1956). In South Africa, Alexander (1957) referred to RVFV isolated from mosquitoes that were reared from eggs collected in a pan. This finding was overlooked for almost three decades and pre-dates any other demonstration of transovarial transmission of any other virus in mosquitoes by sixteen years (Linthicum, *et al*., 1985).

The "virgin-soils" RVF outbreak in the Arabian Peninsula in 2000 appeared to have originated from the large RVF epidemic in East Africa in 1997-1998, based on genomic data (Shoemaker, *et al*., 2002; Bird, *et al*., 2007). The method of introduction of the virus remains unknown (Shoemaker, *et al*., 2002). However, it may be that the introduction of RVFV-infected animals for slaughter served as the source of the infection, as was the case in the Egyptian outbreaks (Hoogstraal, *et al*., 1979; Meegan, 1981; Sellers, *et al*., 1982; Gad, *et al*., 1986).

While the forest endemnicity theory cannot be completely dismissed, it is currently postulated that in sub-Saharan Africa the virus is maintained in inter-epidemic periods by transovarial transmission in aedine mosquitoes, with low level transmission to livestock, and that epidemics are brought about by abnormally heavy rain which causes sudden massive increases in vector numbers (Linthicum *et al*., 1985).

In North and West Africa outbreaks in recent years suggest a different epidemiology to that in sub-Saharan Africa. Epidemics occurred in the absence of heavy rainfall and in arid areas, in association with vectors which are thought to breed in large dams and rivers (Turell, *et al*., 1988; Formenty, *et al*., 1992).

Flying insects (possibly including aedine mosquitoes) can be carried over long distances at high altitudes by strong winds (Sellers, *et al*., 1982). For a few days prior to the 1977 RVF epidemic in Egypt, it was noted that such conditions existed, which could account for the mosquitoes being blown 450-500 km from a focus where the disease had occurred previously (northern Sudan to the Ashwan area in southern Egypt). This could have played a role in the outbreak and must be considered another possible mechanism for the spread of RVFV.

The introduction of RVFV-infected cattle and sheep by transportation on the Nile River, or overland, is considered the most likely cause of the Egyptian outbreaks (Meegan, 1981). Many of these animals came from infected areas in northern Sudan to markets in southern Egypt. Transportation of slaughter animals by sea could account for the antibodies to RVFV detected in the north of Egypt and in eastern coastal towns (Hoogstraal, *et al*., 1979; Meegan, 1981; Sellers, *et al*., 1982; Gad, *et al*., 1986).

Another possible way of transmission could be via persistence of the virus in animal organs. RVFV has been shown to persist for long periods in various sheep organs, in particular the spleen; up to 21 days post infection (Yedloutschnig, *et al*., 1981). Even though some routes of transportation can take a long time in relation to the course of infection, the disease can still persist in infected animals during this period. The same could be true for goats and cattle, and perhaps even camels (Swanepoel and Coetzer, 2004).

Results from a more recent study in wildlife in South Africa on the seroprevalence of RVF in buffalo suggest that buffalo may play a role in the epidemiology of RVF during inter-epidemic periods (Fagbo, *et al*., 2014). This supports an earlier study on buffalo in which 21% (115/550) of animals tested seropositive for RVFV and importantly 7% (9/126) of resampled animals which were initially seronegative, seroconverted during periods outside any reported regional RVFV outbreaks (LaBeaud, *et al*., 2011). These findings highlight the potential importance of wildlife as reservoirs for RVFV and interepidemic RVFV transmission.

It is clear that, even though the inter-epidemic periods of RVF disease are still poorly understood, the virus has crossed significant physical barriers in the past and further new introductions of the virus can be expected in previously unaffected areas. This is especially true considering the ongoing and ever increasing movement of people, animals and mosquitoes, along with changing weather patterns. In South Africa, the boom in wildlife sales and wildlife

translocations over the past ten years may add considerably to this risk since animals are not tested for RVFV before relocation. The definitive existence of a wild mammal reservoir for RVFV has not yet been demonstrated, and for now, on continental Africa, the virus is believed to be maintained by low level circulation among domestic and wild ruminants and via vertical transmission by *Aedes* mosquitoes (Olive, *et al*., 2012).

2.3.3 Hosts

Two key factors that affect the morbidity and mortality during RVF outbreaks include the virulence of the strain of virus and the susceptibility of the vertebrate hosts (Tomori, 1979; Peters and Anderson, 1981; Anderson and Peters, 1988). For practical reasons susceptibility to RVF has always been defined in terms of liability to disease or death. However, this does not always correlate to the minimum dose required to cause infection; for example, lambs suffer more severe disease than monkeys but require a slightly larger dose to become infected (Easterday, 1965; Peters and Anderson, 1981).

It has been suggested that sheep and cattle breeds exotic to Africa are more susceptible to infection compared to indigenous breeds (Davies, 1981). It has been demonstrated that West African and Egyptian sheep are highly susceptible to experimental infection (Tomori, 1979; Olaleye, *et al*., 1996). In addition, many indigenous livestock were affected severely by RVF during epidemics in 1973 in Sudan, 1977-1978 in Egypt and 1987 in West Africa (Matumoto, *et al*., 1950; Eisa, *et al*., 1977; Ksiazek, *et al*., 1989).

Species affected by the virus are numerous, but humans and ruminants are the primary hosts. Species exhibiting extreme susceptibility (70-100% mortality) include lambs, kids, puppies, kittens and some rodents, while those exhibiting high susceptibility (20-70% mortality) include sheep and calves. Species exhibiting moderate susceptibility (less than 10% mortality) include cattle, goats, African buffalo (*Syncerus caffer*), Asian buffalo (*Bubalus bubalis*) various Asian and South American monkeys and humans. Inapparent infection (resistant to infection) is seen in camels, equids, pigs, dogs, cats, African monkeys, baboons and certain rodents. Species not susceptible at all include birds, reptiles and amphibians (reviewed by Swanepoel and Coetzer, 2004).

2.3.4 The role of wildlife

In Kenya, no RVFV antibody was found in African buffalo sera, but there was a low prevalence of antibody in sera from some antelope species (Davies, 1975; Davies and Karstad, 1981).

In South Africa, it was noted during the 1950-1951 epidemic that abortions occurred in springbok (*Antidorcas marsupialis*) and blesbok (*Damaliscus pygargus phillipsi*) antelope, but this was not confirmed as being due to RVFV infection (Joubert, *et al*., 1951). In Zimbabwe a low RVFV antibody prevalence was detected in African buffalo and a few species of antelope, but no record was made of disease (Swanepoel, 1990).

Serologic evidence of RVFV was found in a giraffe (*Giraffa camelopardalis*) during an epidemic period (Bird, *et al*., 2008; Olive *et al*., 2012) and also during an inter-epidemic period (Evans, *et al*., 2008; Olive *et al*., 2012). These authors also demonstrated the same in the desert warthog (*Phacochoerus aethiopicus*).

In the RVF epidemic of 2010 in South Africa multiple indigenous wildlife species were affected. The species reported to have shown signs of disease included springbok, blesbok, bontebok (*D. pygargus pygarus*), waterbuck (*Kobus ellipsiprymnus*), African buffalo, sable (*Hippotragus niger*), kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angasii*) and gemsbok (*Oryx gazella*). Some exotic species that were affected were fallow deer (*Cervus dama*), llama (*Lama glama*), alpaca (*Lama pacos*), Asian buffalo and ibex (*Capra ibex*). In most of these indigenous and exotic wildlife species these were the first documented cases of RVF in the species (Directorate of Animal Health 2012).

The African savannah buffalo (*Syncerus caffer*) has previously been shown to be susceptible to RVFV. In 1996, neutralizing antibodies to RVFV were found in the sera of yearling buffalo from the Kruger National Park (KNP) on the northeastern border of South Africa, implying that there had been recent virus activity in the park (Beechler, *et al*., 2015). There was another case in Kruger National Park in 1999 in captive buffalo where RVFV was islolated from aborted foetuses (Directorate of Animal Health 2012; Beechler, *et al*., 2015) and an outbreak again occurred in 2008 in buffalo on private land adjacent to the KNP (Directorate of Animal Health 2012).

During a six year survey in South Africa (November 2000 - July 2006, n = 550), 7% of re-sampled African buffalo (n = 126) showed seroconversion, demonstrating inter-epidemic transmission of RVFV in these animals (LaBeaud, *et al*., 2011; Olive *et al*., 2012). Since no research has been done on the spread of RVFV from African buffalo, it cannot be concluded that these animals serve as reservoirs for the virus during inter-epidemic periods. However, based on the above study and the one previously referred to above by Fagbo, *et al*. (2014), it appears that buffalo may play a role in maintaining RVFV during inter-epidemic periods.

Even though no cases have been recorded in roan antelope to date, the fact that such a wide array of wildlife are affected, and taking into account the close phylogenetic relationship between sable and roan antelope (Matthee and Davis, 2001), it is reasonable to assume that roan are susceptible to RVFV.

Roan antelope (*Hippotragus equinus*) are listed as 'Vulnerable' in the IUCN Red List of Threatened Species (2004), making such research potentially significant in terms of the long-term survival of this species in the wild. A taxon is listed as 'Vulnerable' when the best available evidence indicates that it meets any of several defined criteria, and it is therefore considered to be facing a high risk of extinction in the wild (Red Data Book, 2004).

In addition, both "disease-free" buffalo and roan antelope are financially very valuable in South Africa. This means their susceptibility to RVF and protection against the disease have important economic implications.

2.4 Diagnosis

While a presumptive diagnosis can be made based on clinical signs (abortions in sheep, cattle and goats) and fatal disease, after periods of high rainfall, more specific diagnosis is required for a disease of this severity. Use is made of various laboratory tests such as serological and molecular assays, but traditionally confirmatory diagnosis still required virus isolation and/or histopathology (OIE, 2014).

A further important consideration is the safety of personnel working with these samples due to the zoonotic potential of RVF and the virus being considered a potential bioweapon. As a result,

the laboratory diagnosis of RVF is restricted to a limited number of reference laboratories worldwide.

2.4.1 Pathological examination

Typical pathological findings include disseminated intravascular coagulopathy, resulting in fibrin thrombi in the liver, spleen, kidneys and lungs; marked hepatic changes/lesions, oedema and haemorrhage in the gall bladder wall (these lesions are most notable in new born animals or aborted foetuses); haemorrhage and oedema in the abomasal folds; mild to moderate splenomegaly; lymph node enlargement, oedema and petechiae; widespread subcutaneous, serosal and visceral haemorrhages; effusion of fluid into body cavities; lung congestion and oedema and enlarged adrenal glands (Swanepoel and Coetzer, 2004).

Tissue specimens submitted for histopathology must include liver, spleen, lymph nodes and kidney. Lesions are characterized by hepatic necrosis. The pathognomonic lesions being primary foci of hepatic necrosis, their frequency varies depending on the species and age of the animal. The liver lesions in lambs are especially pathognomonic (Coetzer, 1977; Coetzer, 1982). Many animals also exhibit lung congestion, alveolar and interstitial oedema, haemorrhages, and some fibrin thrombi in alveolar walls, emphysema, and neutrophil infiltrations (Coetzer 1982)

2.4.2 Virus isolation

RVFV is isolated readily from serum or whole blood during the febrile stage of the disease, as well as from liver, spleen and brain of fresh carcasses or aborted foetuses (Anderson, *et al*., 1989). RVFV can readily be grown in-vitro in mammalian and insect cell cultures, embryonated chicken eggs and variety of laboratory animals. However, some strains of rats, guinea-pigs, chickens and rabbits are resistant (Easterday, 1965; Shimshony and Barzilai, 1983; Meegan and Bailey, 1989). Virus isolation can thus be achieved in hamsters, mice and various cell cultures, including African green monkey kidney cells (Vero), baby hamster kidney (BHK-21) CER, mosquito cell lines, primary lamb, calf and goat kidney or testis cells (reviewed by Swanepoel and Coetzer, 2004; Gerdes, 2004). These procedures are, however, expensive and time consuming and hence often not adequate for RVF outbreaks and regulatory purposes.

2.4.3 Serological assays

Viral antigen can often be rapidly detected in impression smears of infected tissue using immunofluorescence (Pini, *et al*., 1970) and in tissue sections by employing immunoperoxidase staining (Swanepoel, 1976; Swanepoel, 1981; Swanepoel, *et al*., 1986.). Enzyme linked immunosorbent assay (ELISA) and reversed passive haemaglutination can also be employed to demonstrate antigen in serum (Niklasson, *et al*., 1983; Meegan, *et al*., 1989; Peters, *et al*., 1989). ELISAs designed for detection of antibodies to RVFV in domestic ruminants, wild ruminants and humans have been described previously (Paweska, *et al*., 2003a, Paweska, *et al*. ,2003b, Paweska, *et al*., 2003c, Paweska, *et al*.,2005a, Paweska, *et al*., 2005b, Paweska, *et al*., 2007, Paweska, *et al*., 2008).

The IgG sandwich ELISA is more sensitive in detection of the earliest immunological responses to infection or vaccination with RVFV, when compared to serum virus neutralisation (SNT) and haemagglutination-inhibition tests (Paweska *et al*. 2003a). Its sensitivity and specificity derived from field data sets ranged in different ruminant species from 99.05 to 100%. In comparison, specificity of the IgM-capture ELISA varied between different species from 97.4 to 99.4%; while its sensitivity was 100% in sheep tested 5–42 days post-infection. The results of the study, based on field-collected, experimental and post-vaccination sera, indicate clearly that these assays are very useful for epidemiological surveillance, control programmes, import/export veterinary certification, early diagnosis of infection, and for monitoring of immune response in vaccinated animals (Paweska *et al*. 2003a).

ELISAs can be employed to confirm the presence of either specific IgM antibodies, which appear transiently from 4 days after infection or specific IgG antibodies, which appear from 8 days after infection and may persist for several years (Paweska, *et al*., 2005b). The detection of IgM would suggest a current or recent infection. However, IgG-based ELISAs cannot distinguish between past and current infection unless paired serum samples are analysed (acute and convalescent) and a four-fold increase in antibody titre observed (Mansfield *et al*., 2015).

The ELISA test has been shown to be extremely reliable and accurate for monitoring of immune response in vaccinated animals of various domestic species (Paweska *et al*., 2003a). However, the same authors concluded that time-dependent (time since infection) changes in the sensitivity of the assay were clearly evident in the study, and especially for IgM-capture

ELISA: the test was 100% sensitive 5–42 days post-infection, and only 12.5% sensitive 3 weeks later. This may account for some negative results more than 21 days post vaccination. However, in another study, Paweska *et al*. (2008) demonstrated that the rNp-based IgG I-ELISA is robust, has high diagnostic accuracy, and can be used reliably for diagnosis, import–export veterinary certification and for seroepidemiological studies of RVFV infections in African buffalo.

The SNT has been used to detect antibodies against RVFV in serum of a variety of species, and the OIE considers this test as the gold standard to test for RVFV antibodies (OIE 2009). The test is highly specific, but it can only be performed with live virus and therefore is not recommended for use outside endemic areas or in laboratories without appropriate biosecurity facilities and vaccinated personnel (OIE 2009). Most types of cell cultures including Vero, BHK-21 and rhesus monkey kidney epithelial cells can be used in the SNT test for RVF (WHO 1982).

In a trial in Senegal on 267 small ruminants, half were vaccinated with Clone 13 vaccine and showed seroconversion using ELISA and SNT in more than 70% of the animals, starting on Day 60. The seropositivity rates remained high throughout the observation period, including on the last study day, when more than 70% of the sheep and goats had neutralising antibody titres above the positivity threshold. Antibody levels persisted up to 1 year after vaccination (Lo, *et al*., 2015).

In the trials using Clone 13 conducted by Dungu, *et al*. (2010) on sheep, very good neutralising antibody responses were recorded in all vaccinated sheep, from Day 7 post-vaccination and a dose–response effect was clearly evident. Lambs born from vaccinated ewes also showed significant antibody titres. The very good seroconversion recorded in these trials, with up to four-fold increases in neutralising antibody titres, as early as Day 7 post-vaccination, indicated a good protection against RVF. This was substantiated by the subsequent challenge results where even the few individuals with low titres still showed resistance to disease challenge.

In the trials on cattle conducted by Von Teichman, *et al*. (2011), four of five calves vaccinated with Clone 13 vaccine showed an antibody response of 1:4–1:16 by Day 7, which peaked by Day 14 with an antibody titre of 1:256 in one animal. By Day 14 post-vaccination, the 5th animal had also seroconverted. Antibody titres decreased over the following two weeks in 3

out of 5 animals, but increased in the remaining two (Day 28). When challenged with virulent RVFV, titres increased to > 1:512 within seven days of challenge and remained high for the remainder of the animal trial (Day 56), as a result of an anamnestic antibody response.

Njenga *et al*. (2015) concluded that RVF Clone 13 vaccine is safe to use and has high (>90%) immunogenicity in sheep and goats but moderate (> 65%) immunogenicity in cattle. This was based on a blind randomized controlled field trial involving 404 animals (85 cattle, 168 sheep, and 151 goats) of which 194 were vaccinated and the rest vaccinated with placebo. Animals were monitored for one year and virus antibodies titres assessed on days 14, 28, 56, 183 and 365. Vaccinated cattle (N = 42) did not develop any anti-RVFV IgM antibodies but 67% developed anti-RVFV IgG antibodies. These were maintained in 43.6% of the animals through Day 365 post-vaccination. In vaccinated goats (N = 72), 72% developed IgM antibodies and 97% developed IgG antibodies that were maintained in all animals through Day 365. In vaccinated sheep (N = 78), 84% developed IgM and 91% IgG antibodies that were maintained in 52.6% of the animals by Day 365.

At Day 14 post vaccination, the chance of being seropositive for IgG in the vaccinated group was significantly higher ($P < 0.05$) in vaccinated animals compared to the placebo group, with an odds ratio of 4 in cattle, 90 in goats, and 40 in sheep. In contrast, the IgM antibody response between placebo and vaccinated groups in cattle and goats was statistically insignificant. However, in sheep, the IgM response was significantly higher in the vaccinated as compared to placebo group of animals. The need for caution when interpreting ELISA results in different species and when interpreting titres in vaccinated animals was emphasized by Njenga *et al*., 2015.

To determine whether the IgG antibodies in this study were neutralizing, a virus neutralisation test (VNT) was run for vaccinated bovine (N = 40), goats (N = 46), and sheep (N = 46) sera that were ELISA positive. Overall, 89.6% (116/130) of the ELISA positive samples were also positive by VNT whereas 91% (10/11) of the ELISA negative samples were negative by VNT. Cattle had lower ELISA and VNT titres whereas goats had the highest titres (Njenga *et al*. 2015).

Even though there was no natural RVF outbreak during the period of the above-mentioned trial, the Clone 13 vaccinated animals developed high neutralizing antibodies. The authors reported

that their results were in agreement with the trials carried out by Dungu *et al*. (2010) and Von Teichman *et al*. (2011), where neutralizing antibodies and prevention from challenge with virulent virus were reported in all vaccinated animals.

There are no similar trials that have been conducted on wild ruminants.

The OIE lists both the ELISA and SNT as the recommended method for detecting an immune response in populations considered free from disease, as well as post-vaccination to detect an immune response (OIE, 2010).

2.4.4 Molecular assays

Reverse transcription-polymerase chain reaction (RT-PCR) can be used to readily detect viral nucleic acid in serum and other tissue from infected humans, livestock and mosquitoes (Ibrahim, *et al*., 1997; Garcia, *et al*., 2001; Drosten, *et al*., 2002; Jupp, *et al*., 2002; Sall, *et al*., 2002). Highly sensitive RT-PCR assays for the detection and quantification of RVFV have been developed. These include real-time RT-PCR (rt RT-PCR) (Ibrahim, *et al.*, 1997; Garcia, *et al*., 2001; Sall, *et al*., 2002), including those based on TaqMan probe technology (Drosten, *et al*., 2002; Bird, *et al*., 2007). More recently real-time RT loop-mediated isothermal amplification assays (RT-LAMP) targeting the large RNA segment were developed and evaluated for the detection of a wide spectrum of RVFV isolates and clinical specimens (Peyrefitte, *et al*., 2008; Le Roux, *et al*., 2009). The latter study reported a 100% agreement between the RT-LAMP, TaqMan based RT-PCR and virus isolation results (Le Roux, *et al*., 2009). The RT-LAMP assay had a very high diagnostic sensitivity and specificity when used on a variety of clinical specimens from humans and animals and detection of specific viral genome targets in positive clinical samples takes less than 30 minutes. In addition, the equipment required to run the test is simple and relatively inexpensive, giving it even more practical applications in resource-poor and remote areas of outbreaks (Peyrefitte, *et al*., 2008; Le Roux, *et al*., 2009).

2.5 Control

Control of RVF is either achieved through vector control, limiting exposure to infected vectors or via immunization.

2.5.1 Vector control

Vector control measures include burning grass cover and larvacide treatment at strategic times in dambos (prime aedine mosquito breeding grounds) to reduce the viability of mosquito eggs and thus suppress overall mosquito numbers (Logan, *et a.l*, 1990; Whittle, *et al*., 1993). Other measures include chemical control of adult mosquitoes, moving stock to well-drained wind swept pastures (usually at higher altitudes) or confining stock to mosquito-proof stables, all of which are somewhat impractical and not very effective, except as an aid during an outbreak of disease (Swanepoel and Coetzer, 2004). These measures are especially not practical for wildlife species which cannot be contained and often roam large areas.

2.5.2 Vaccination

Immunization remains the only effective way to protect livestock and wildlife from RVF infection. Several vaccines exist: the Smithburn live attenuated virus vaccine, formalin-inactivated RVF vaccines, Clone 13 live attenuated virus vaccine and a MP-12 live attenuated virus vaccine.

The Smithburn strain (mouse neuro-adapted) of RVF has been used at different passage levels to produce livestock vaccine in South Africa and Kenya since the 1950's, while wild strains of RVFV are used to prepare formalin-based inactivated cell culture vaccines in South Africa and Egypt. In South Africa, the original Smithburn vaccine was modified several times and since 1971 has been propagated in BHK-21 cells for preparation of freeze dried vaccine (Kaschula, 1953; Weiss, 1957; Weiss, 1962; Barnard and Botha, 1977; Barnard 1979).

The modified live Smithburn vaccine can be produced easily in large quantities, is inexpensive and gives strong immunity in sheep within 6-7 days post single inoculation. However, a proportion of pregnant ewes may abort or undergo teratology of the foetus and hydrops amnii and prolonged gestation (Coetzer and Barnard, 1977). This indicates that the virus is only

partially attenuated and should only be used in pregnant animals in an epidemic where the possible adverse effects are outweighed by the risks of not vaccinating (Davies, 1981).

In contrast to this, the formalin-inactivated RVF vaccines are safe to use in pregnant animals, but are expensive to produce and only induce a short period of immunity, thus necessitating regular booster vaccinations in order to ensure adequate protection (Caplan, *et al*., 1985).

A mutagen-derived MP12 candidate vaccine virus was tested and shown to be safe for use in pregnant cattle and sheep, even when inoculated directly into bovine foetuses (Hubbard, *et al.*, 1991; Baskerville, *et al*., 1992; Morrill, *et al*., 1997; Morrill, *et al*., 1997). The flaw in these trials was the fact that the vaccines were administered to animals already beyond the first stage of pregnancy, meaning organogenesis had already taken place and foetuses were no longer susceptible to the teratogenic effects of attenuated viruses. When the vaccine was administered to sheep at an earlier stage of pregnancy, 14% showed teratology (Hunter, *et al*., 2001). This vaccine is not in commercial production.

RVF Clone 13 is a recently developed vaccine and available commercially. The 74HB59 strain of RVFV was isolated from a human case in the Central African Republic and shown to be composed of a heterogeneous population of viruses (Muller, *et al*., 1995). One of these clones, C13, was of particular interest in that it proved to be avirulent in mice and hamsters, and highly immunogenic. Due to a large deletion in the NSs protein encoding gene of the small S segment of the genome, this is an avirulent RVFV named Clone 13. The virus grows to high titres in cell cultures and due to the deletion in the sequence coding for the virulence factor, it is unable to revert back to virulence, making it an attractive candidate for vaccination. Vaccination trials in sheep and bovines elicited a high antibody response protecting against RVFV challenge, without any deleterious effects. In this study, pregnant ewes never aborted (87.5% of individuals protected against abortion on challenge with wild virus) and there was no teratogenesis (Swanepoel and Coetzer, 2004; Albarino, *et al*., 2007; Dungu, *et al*., 2010).

In a study to evaluate the efficacy and safety of the Smithburn (live attenuated) and Clone 13 vaccines in calves, it was concluded that both vaccines produced a good neutralizing antibody response protecting against RVFV challenge, and no viraemia could be detected post challenge (Von Teichman, *et al*., 2011). Njenga *et al*. (2015) concluded that RVFV Clone13 vaccine is

safe to use and has high (> 90%) immunogenicity in sheep and goats but moderate (> 65%) immunogenicity in cattle.

Persuading farmers to rigorously follow a vaccination protocol in the absence of regular disease is always challenging. The fact that RVF epidemics occur at irregular intervals with long inter-epidemic periods, mean it is even more difficult to persuade farmers to vaccinate routinely against this disease. In addition, RVF epidemics are hard to predict and have a sudden onset. This has in the past lead to vaccine availability issues/shortages during RVFV outbreaks. Taking this, the nature of the disease, and the availability of the vaccine into account, it is generally recommended in African countries with large sheep and goat populations to regularly immunize the offspring of vaccinated ewes and nannies at six months of age (Assad, *et al*., 1983). This is when colostral immunity has waned and a single dose of Smithburn vaccine should afford life-long protection (Assad, *et al*., 1983).

In cattle, the Smithburn RVF vaccine induces a poor antibody response and hence it is preferable to vaccinate them with the formalin-inactivated vaccine (Barnard and Botha, 1977; Barnard, 1979). This ought to ensure that cows transfer colostral immunity to their calves. Cattle should also be given a second vaccine 3-6 months after the first, and then receive annual vaccinations, before the rainy season since immunity only lasts approximately one year (Barnard and Botha, 1977).

Despite the above research, the current data sheet for use of the inactivated RVF vaccine (formalinised RVF virus) recommends vaccinating cattle, sheep or goats as follows:

Animals susceptible to disease can be immunised at any age. This is irrespective of the stage of pregnancy and lactation. Calves and lambs born from animals with immunity can only be effectively immunised after the age of six months to ensure that acquired maternal antibodies have waned. In order to obtain optimal protection, a booster vaccination is recommended 3 - 4 weeks after initial vaccination. Annual vaccination is recommended, this is best done in the late winter or early spring (Data sheet, Inactivated RVF vaccine for cattle, sheep and goats, Onderstepoort Biological Products:

https://www.obpvaccines.co.za/Cms_Data/Contents/OBPDB/Folders/Product/~contents/GZC2QWWS33BNW7VE/2146%20Inactivated%20RVF_PI.pdf).

The current data sheet for the freeze-dried, live attenuated RVF (Smithburn strain) recommends vaccinating cattle, sheep and goats as follows: Animals susceptible to disease can be immunised at any age. Calves and lambs born from animals with immunity can only be effectively immunised after the age of six months to ensure that acquired maternal antibodies have waned. Since disease outbreaks typically occur during the late summer and autumn, it is recommended to vaccinate susceptible animals during spring. This should be three to six weeks before the mating season. It is advisable to plan breeding so that lambing occurs between February and April to ensure that lambs will be adequately protected by maternal antibodies during high risk times of the year. Complete immunity is normally achieved three weeks after inoculation. Annual vaccination is recommended (Data sheet, Live RVF vaccine for cattle, sheep and goats, Onderstepoort Biological Products:

https://www.obpvaccines.co.za/Cms_Data/Contents/OBPDB/Folders/Product/~contents/X5Q2767Q298MMNA3/2153_RVFLive_PI.pdf).

The freeze-dried, live attenuated RVF (Clone 13 strain) for the immunisation of cattle, sheep and goats against RVF, is recommended to be used as follows, according to the data sheet: Since disease outbreaks typically occur during the late summer and autumn, it is recommended to vaccinate susceptible animals during spring or early summer. Animals may safely be vaccinated from 2 months of age, unless their mothers were immunised, in which case they should be vaccinated after the age of six months to ensure that acquired maternal antibodies have waned. The vaccine can safely be used in pregnant animals. Most animals will be immune three weeks after vaccination. Annual vaccination is recommended for optimal immunity (Data sheet, RVF Clone 13 vaccine for cattle, sheep and goats, Onderstepoort Biological Products:

https://www.obpvaccines.co.za/Cms_Data/Contents/OBPDB/Folders/Product/~contents/AME2WHYSW2LJB4WB/2213_RVF%20CLONE13_PI.pdf).

Based on the above findings regarding safety and efficacy of Clone 13 vaccine, and availability issues with all vaccines during outbreaks, Clone 13 RVF vaccine was selected as the best candidate for the trial. This is despite the fact that to date there is no published research with regard to the use of any RVF vaccine in wildlife, nor are there any recommended vaccine protocols for wildlife.

3. CHAPTER 3: JUSTIFICATION AND AIM OF THIS STUDY

It is evident from a review of the available literature that RVF occurs fairly regularly in South Africa. It not only affects domestic animals, but a large number of species including multiple wildlife species, often with dire consequences. It is thus a very important disease for both domestic and wild animals. In view of severe outbreaks of RVF in recent years in Africa (and South Africa in particular), the high commercial value of wildlife, and the endangered/threatened status of many wildlife species, protection against RVF infection is essential.

Looking more broadly it is clear that the threat from RVF should not be taken lightly as evidenced by outbreaks in new areas of the world – Egypt in 1977, West Africa in 1988, and the Arabian Peninsula in 2000 (Pepin, *et al*., 2010) and the re-emergence of the disease after periods of long absence. Competent vectors occurring in countries previously free of these, high viral titres in viraemic animals, global climate changes and international travel and trade in livestock all make this threat more credible.

As discussed above vaccination is considered the most effective and reliable way to protect animals and in addition is the only practical option for disease prevention in wildlife at this time. Clone 13 RVF vaccine is the newest and most promising vaccine with regards to safety and efficacy, as evidenced by studies cited above, and was thus chosen as the best vaccine option for use in wildlife. In addition, it is more readily available than some of the other vaccines, and has not been tested on wildlife.

The aims of the research are:

To determine whether Clone 13 RVFV vaccine is effective in creating a humoral immune response to RVF vaccine in the African buffalo (*Syncerus caffer*) and roan antelope (*Hippotragus equinus*).

To determine whether vaccination results in any obvious clinical side effects, such as general malaise or abortion.

4. CHAPTER 4: MATERIALS AND METHODS

4.1 Experimental Design

The buffalo used in this project belonged to East Cape Parks and Tourism Agency and were part of the extensive population on the Great Fish River Nature Reserve, managed by the agency. The park is situated close to Fort Brown in the Eastern Cape Province of South Africa. The collection of samples and vaccination of the buffalo coincided with planned chemical capture and subsequent relocation of animals as part of sales/management plans. Animals were sampled and vaccinated at the same time without additional/undue stress/risk to them. Due to the value of these animals and risks associated with chemical immobilisation, sampling intervals did not follow theoretical research models, but were undertaken as opportunities presented, i.e immobilisations for mandatory disease testing requirements and relocation. There were some complications with the sale of some animals which meant the disease testing had to be repeated, providing for further sampling opportunities. This was in line with permission granted by the owner of the animals, East Cape Parks and Tourism Agency.

The roan antelope were part of an extensive herd resident on a privately-owned game reserve in the greater Bedford area, Eastern Cape Province of South Africa. They were relocated post capture to a private game reserve in the Janssenville area of the Eastern Cape Province, which was where the second and third immobilisation for sample collection took place. The initial collection of samples and vaccination of the roan antelope co-incided with planned chemical capture and subsequent relocation of these animals as part of the sale. Thus, animals were sampled and vaccinated at the same time without additional/undue stress/risk to them. The repeat immobilisation and sampling were however undertaken for the primary purpose of collecting further/follow-up samples, with the owner's consent. Due to the extensive nature of the terrain on the new reserve, only a limited number of animals could be darted successfully.

4.2 Samples collected

Twenty-four buffalo were captured on 15 and 16 April 2011 (Table 1). EDTA and serum samples were collected for the purpose of running SNT and ELISA to test for antibodies specific to RVFV from all animals, except for animal number six. Blood samples were collected from the jugular vein using vacutainer needles, hubs and blood tubes. Additional

blood samples were taken and submitted randomly for further laboratory analysis (haematology and biochemistry). A blood smear was made from the additional submitted samples. Further blood samples to test for response to the RVF Clone 13 vaccine and random biochemistry and haematology evaluation were subsequently collected in a similar manner from all animals, at different intervals, as outlined in Table 1.

The nine roan antelope captured on 14 March 2011, were blood sampled in a similar manner (Table 2). Additional blood samples were taken and submitted randomly for further laboratory analysis (haematology and biochemistry). A blood smear was made for examination from the additional submitted samples. A small number of animals were immobilised multiple times and sampled in the same manner on 11 April (animals 3, 5, 6 and 9) and 24 October 2011 (animals 5, 6 and 7) (Table 2).

Table 1: Buffalo age estimates (at time of capture), sex, and immobilisation/sample collection dates.

No.	Sex	Estimated Age (at capture)	Immobilisation and sample collection dates during 2011						
			15and16 April	26 May	23 June	28 June	12 July	1 September	15 November
1	M	18 months	X					X	X
2	M	18 months	X					X	X
3	F	2,5 years	X					X	X
4	F	Adult, pregnant	X					X	X
5	F	18 months	X					X	X
6	F	12 months						X	X
7	F	Adult	X					X	X
8	F	Mature	X	X		X			
9	F	18 months	X	X		X			
10	M	Adult	X	X		X			
11	F		X	X		X			
12	F		X	X		X			
13	F	Adult	X	X				X	X
14	M	Adult	X	X		X			
15	F	Adult	X	X		X			
16	F	15 months	X	X	X				
17	F	Adult	X	X	X				
18	F	12 months	X	X			X		
19	F	Adult	X				X		
20	F	Adult	X	X			X		
21	F	Mature	X	X			X		
22	F	Adult	X					X	X
23	M	15 months	X					X	X
24	M	Adult	X	X			X		
25	F	Born 20.4.2011 in the boma							X *

Note: M = Male, F = Female; * = unvaccinated

Table 2. Roan age estimates (at time of capture), sex, and immobilisation/sample collection dates.

No.	Sex	Estimated Age (at time of capture)	Immobilisation and sample collection dates during 2011		
			14 March	11 April	24 October
1	F	± 3 years	X		
2	F	± 3 years	X		
3	F	?	X	X	
4	F	2 years	X		
5	F	8-10 years	X	X	X
6	F	10-12 years	X	X	X
7	F	± 6 years	X		X
8	M	± 5 years	X		
9	F	Old, ±12 years	X	X	

Note: M = Male, F = Female

Samples were kept at 4°C after collection and the serum samples centrifuged for 10 minutes before they were transported to the relevant laboratories. A serum and EDTA sample were sent as soon as possible after an animal was immobilisied by courier to IDEXX Laboratories in Port Elizabeth. A second serum sample was sent by courier on ice to the Department of Veterinary Tropical Diseases (DVTD), Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Pretoria.

4.3 Vaccine administration

After immobilisation/capture, blood was collected from each animal (as above) and then 1 ml of Clone 13 RVFV vaccine (Onderstepoort Biological Products, South Africa) was administered by subcutaneous injection, according to the recommendations of the manufacturer for the vaccination of cattle, sheep and goats. This was administered to most animals over the

left lateral shoulder for consistency and practical reasons. Vaccine was kept on ice in a cooler during field work to ensure its integrity. Other routine treatments are detailed below and should have no bearing on this study.

4.4 Experimental animals used

4.4.1 Buffalo

On 15 and 16 April 2011, a total of 24 buffalo were immobilised for East Cape Parks Board and placed in a boma on the Great Fish River Game Reserve for disease testing and sale. The animals were darted from a helicopter (Robinson R44) using a "Pneudart' dart gun and darts. The buffalo were immobilised using a combination of etorphine ("M99" 9.8 mg/ml, Novartis Animal Health), or thiafentanil ("A30-80" 10 mg/ml, Wildlife Pharmaceuticals), with azaparone ("Stresnil" 40 mg/ml, Janssen Pharmaceuticals) and hyalase (Kyron Pharmaceuticals, 5000 IU per vial). The difference in immobilisation protocols is due to drug availability at the time. Some animals were treated with ketamine HCl (compounded 1g powder per vial, Kyron Pharmaceuticals, South Africa) in order to make them calmer and easier to handle post immobilisation. Other animals received small doses of medetomidine (compounded, 20 mg/ml, Kyron Pharmaceuticals, South Africa) for the same reason, or in some cases to prolong the immobilisation for transport, without administering more opioid drugs. The medetomidine was reversed with the alpha-2 antagonist, yohimbine (compounded 6.25 mg/ml, Kyron Pharmaceuticals, South Africa). A small number of animals received butorphanol to improve respiration (compounded, 50 mg/ml, Kyron Pharmaceuticals, South Africa). All animals were treated with routine treatments for buffalo post darting, namely: a long acting antibiotic to prevent infection at the dart wound or systemically post the stress of capture - either a long-acting penicillin (either "Peni LA", Virbac Pharmaceuticals, or "Lentrax", Merial South Africa), or a long acting oxytetracycline (either "Terramycin" or "Liquimycin", Zoetis South Africa). The difference in antibiotics used was due to availability on the day, all being considered effective in this situation. All animals also received a multivitamin to support the immune system due to relocation stress and address any deficiencies ("Multivite", Norbrook (Biotech Vet)), ivermectin to treat internal and external parasites ("Dectomax", Pfizer South Africa), and a tranquiliser in the form of haloperidol (compounded 20 mg/ml, Kyron Pharmaceuticals, South Africa).

All animals were vaccinated against RVF using the Clone 13 vaccine ("RVF Clone 13", Onderstepoort Biological Products, South Africa), by sub-cutaneous injection of 1 ml of the vaccine according to the recommendations of the manufacturer for the vaccination of cattle, sheep and goats. All animals were reversed from chemical immobilisation with the antidote naltrexone, at the recommended dosage ("Trexonil" 50 mg/ml, Wildlife Pharmaceuticals) and recovered in bomas (holding pens) without incident.

A female calf was born in the bomas on 20 April but was not vaccinated due to age. A blood sample was collected from her during the November sampling (Table 1; indicated with *).

Subsequent to the capture, these 24 animals were immobilised several times for disease testing or for sale/movement, as detailed in Table 1. The above protocol was used with some minor differences – the animals were darted on foot since they were already in the boma, in some cases a different brand of anitibiotic was used, and some animals were treated with diprenorphine hydrochloride ("M50-50" 12 mg/ml, Novartis Animal Health, South Africa) and naltrexone (Naltrexone 40 mg/ml, compounded, Kyron pharmaceuticals) to reverse the immobilisation. In addition, some animals were treated on occasion with flumethrin ("Drastic Deadline", Bayer Animal Health) for external parasite control, or abamectin topically ("Buckguard" abamectin 0.5%, Virbac Pharmaceuticals, South Africa) and before relocation to new game reserves, were vaccinated against clostridial disease with 2ml of a multivalent clostridial vaccine ("Supavax", Intervet SA – MSD Animal Health) administered sub-cutaneously. All animals recovered without incident from immobilisation. The RVF Clone 13 vaccine was not repeated, nor was the haloperidol tranquilisation.

All immobilisation protocols and doses were in line with /as described in "Chemical and Physical Restraint of Wild Animals, a training and field manual for African species" (Burroughs, *et al*., 2012).

4.4.2 Roan Antelope

Nine roan antelope were darted on 14 March 2011, on a game reserve in the greater Bedford area, Eastern Cape. The animals were darted from the ground using "Dan Inject" and "Pneudart' dart guns and darts. Animals number one, two, five, six and nine (Table 2) were immobilised with a combination of thiafentanil ("A30-80" 10 mg/ml, Wildlife Pharmaceuticals), azaparone (V-Tech compounded, 100 mg/ml) and hyalase (Kyron Pharmaceuticals, 5000 IU per vial); animals three, four and seven were immobilised with a combination of thiafentanil, etorphine hydrochloride ("M99" 9.8 mg/ml, Novartis Animal Health), azaparone and hyalase. Animal number 8, the herd bull, was immobilised with a combination of etorphine hydrochloride, azaparone and hyalase. The reason different combinations were used was primarily due to drug availability at the time. Some animals were treated with ketamine ("Ketamine – Fresenius" 50 mg/ml, Fresenius Kabi) to make them more manageable post immobilisation. All animals were treated with routine treatments for antelope being relocated, namely: a long acting penicillin to prevent infection at the dart wound or systemically post the stress of capture (either "Peni LA", Virbac Pharmaceuticals, or "Lentrax", Merial South Africa), a multivitamin to support the immune system due to relocation stress and address any deficiencies ("Multivite", Norbrook (Biotech Vet)), ivermectin to treat internal and external parasites ("Dectomax", Pfizer South Africa), an ecto-parasitic agent in the form of flumethrin ("Drastic Deadline", Bayer Animal Health), and a tranquiliser in the form of zuclopenthixol-acetate ("Cloxipol Acuphase" 50 mg/ml, H..Lundbeck (Pty) Ltd, South Africa). All animals were vaccinated against clostridial disease with 2 ml of a multivalent clostridial vaccine ("Supavax", Intervet SA – MSD Animal Health) administered sub-cutaneously in the lateral shoulder area.

They were also vaccinated against RVF using "RVF Clone 13" vaccine (Onderstepoort Biological Products, South Africa) by administering 1 ml sub-cutaneously in the lateral shoulder, according to the recommendations of the manufacturer for the vaccination of cattle, sheep and goats. All animals were reversed from chemical immobilisation with the antidote naltrexone, at the recommended dosage ("Trexonil" 50 mg/ml, Wildlife Pharmaceuticals) and recovered from immobilisation in the transport trailer without incident. The animals travelled and offloaded well.

Subsequent to the capture, animals 3, 5, 6 and 9 were immobilised again on 11 April, and animals 5, 6 and 7 on 24 October 2011, for blood collection, as detailed in Table 2. These immobilisations took place on Meerlust farm near Jansenville, Eastern Cape Province. Only these animals were immobilised since the animals were quite wild and the new habitat difficult, making it impossible to dart more animals from the ground.

The above protocol was used with some minor differences – long acting oxytetracycline ("Terramycin LA" 200mg/ml, Pfizer Animal Health) was added to the treatment protocol since some *Theileria*-like organisms/piroplasms were seen on blood smear post capture, and animals three and six were also treated with ketoprofen ("Ketofen 10%" 100mg/ml, Merial South Africa) for wounds sustained post-relocation which were opened up and treated. The "Supavax" vaccine was repeated in all animals to boost immunity (according to the recommended vaccination protocol). All animals recovered without incident.

All immobilisation protocols and doses were as described in "Chemical and Physical Restraint of Wild Animals, a training and field manual for African species" (Burroughs, *et al*., 2012).

4.5 Laboratory techniques

4.5.1 Haematology and Biochemistry

The blood smears, serum and EDTA samples couriered to IDEXX Laboratories, South Africa, were analysed using standard protocols (as detailed below) to obtain basic haematology and biochemistry information. The blood smear was evaluated with light-field microscopy by a specialist Veterinary Clinical Pathologist. These procedures were carried out to detect any ill or immune-compromised animals, to detect any blood-borne parasites and to monitor possible side effects of the vaccination.

The haematology was performed on Cell Dyn 3500 (Abbott Laboratories, Abbott Park, Illinois U.S.A.) MAPPS laser technology analyser for white blood cell count (WCC), red blood cell count (RBC), haematocrit (HT), haemoglobin concentration (Hb) and platelets. Leukocyte differential counts and blood smear examinations were performed manually using Rapid Romanowski-stained smears at a magnification of X1000. Serum chemistry assays were performed on the Vitros 250 Dry Chemistry Analyser (Ortho-Clinical Diagnostics, Inc.).

Parasitaemia estimates were based on the proportion of parasitized cells encountered during a 100-cell leukocyte differential count and then converted to a red cell percentage by using the proportion of WCC to RCC.

4.5.2 Serum virus neutralisation test

The SNT was done at the SANAS accredited Virology Laboratory of the DVTD according to the standard operating procedure (SOP) in use. The test was performed in 96-well microtitre plates and a vaccine strain of RVFV (obtained from Onderstepoort Biological Products) was used as antigen.

The sera tested were diluted 1:5 in phosphate buffered saline of Dulbecco (PBS+) and inactivated for 30 minutes in a water bath at 56°C. Two-fold dilutions of the serum were prepared in duplicate on the microtitre plate (1-6 wells) and 100 µl of each dilution were used. The RVF stock virus, with a titre of 104.6 tissue culture infectious dose50 (TCID50)/100 µl, was diluted in minimum essential medium (MEM), containing 5% foetal calf serum (Highveld Biological, South Africa) to provide 100 TCID50. A volume of 100 µl was added to each well that contained a diluted test serum. To determine if the correct concentration of the virus was used, a series of ten-fold dilutions of the virus (10-1 – 10-4) were prepared. This virus control (back titration) was used in 3 wells. The plates were incubated at 37°C for 1 h in an incubator with an atmosphere containing 5% CO2. A volume of 80 µl of Vero cells at a concentration of 480 000 cells/ml was added to each well of the microplate including the virus controls, followed by incubation at 37°C in an atmosphere containing 5% CO2. The plates were read once the virus controls showed cytopathic effect (CPE), 10-1 shows 75% CPE, 10-2 shows 50% CPE and 10-3 show 25% CPE. It was important that 10-2 showed 50% CPE because this was the same as the virus titre used in the test (TCID50).

Where results of CPE below 50 % were smaller than expected, half-log dilutions were used to determine more accurate results.

The monolayers were examined daily from day three until the dilution of 10-2 showed 50% CPE. Evidence of CPE was determined by using an inverted microscope and the results were recorded as the last dilution where no inhibition of CPE of the virus could be seen.

4.5.3 ELISA test

An IgM antibody capture ELISA and indirect IgG-ELISA were used according to the SOP at the Agricultural Research Council's Onderstepoort Veterinary Research Institute. The indirect IgG ELISA was published by Paweska *et al*. (2003c). Briefly:

A volume of 50 µl per well of original RVFV antigen and control antigen, each diluted 1/1000 in a carbonatebicarbonate buffer pH 9.6, was used. After incubation at 4°C overnight, unbound antigen was removed by washing the plate three times with 250 µl per well of TST buffer (Tris saline, Tween pH 8 ± 0.2). Thereafter, 100 µl of blocking buffer (used also as a diluting buffer) consisting of 3% fat-free milk powder (Clover SA, Pty Ltd) in TST was added to each well, and the plate incubated for 1 h at 37°C. After washing the plate three times as before, 50 µl of control and test sera, diluted 1:100 in diluting buffer, were added in duplicates to wells pre-coated with positive and control antigen. After incubation at 37°C for 60 min unbound antibody was removed by washing the plates three times with 250 µl per well of TST buffer. A volume of 50 µl recombinant protein G conjugated with horse radish peroxidase (Cat No. 10-1223, Zymed) diluted 1:10 000 was added to each well and the plates incubated at 37°C for 1 h. Unbound conjugate was removed by washing the plates three times with 250 ul per well of TST buffer. A volume of 50 µl substrate/chromogen (TMB, Cat No. 00-2023, Zymed) was added to each plate and the plates incubated in the dark for 20 min at room temperature (22-25°C). Reactions were stopped by adding 50 µl/well of 1 M H2S04 and colour development was immediately assessed in a spectrophotometer (EL 340, Bio-Tek Instruments) using 450 nm and 690 nm reference filters. Optical density (OD) readings were converted to PP values (percentage of strong positive control serum) using the following equation:

% PP = [(Mean OD of test sample, positive antigen – Mean OD of test sample, neg. antigen) / (Mean OD of C++. positive antigen – Mean OD of C++. negative antigen)] x 100

where % PP = Percentage positivity of C++.

The IgM antibody capture ELISA was published by Williams *et al*., (2011). Briefly:

100 µl per well of rabbit antiserum to sheep-chain of IgM (ICN Pharmaceuticals), diluted to 1 µg/ml in PBS, was used as a capture antibody to coat ELISA microtiter plates (Corning Inc.). This was done overnight in a humidity chamber at room temperature. Washing and diluting buffers followed the same protocol as described above for the IgG ELISA. The plates were blocked with 100 µl/well Stabilcoat buffer (Surmodics, Eden Prairie, USA) and incubated for 1 h at room temperature.

Positive and negative control sera and test sera were diluted 1:100 in dilution buffer (6% milk powder in TST buffer) and 100 µl were added per well according to plate layout. Dilution buffer served as the conjugate control. Plates were incubated for 1 h at 37°C and then washed three times with 300 µl TST buffer per well. The nucleoprotein conjugated to horseradish peroxidase (N–HRP conjugate) was diluted 1:10,000 in dilution buffer and 100 µl added per well. The plates were incubated for 60 min at 37°C and washed as before. A volume of 100 µl of a TMB ready-to-use substrate (Zymed, San Francisco, USA) were added to each well and the reaction was allowed to proceed until the OD reached 0.400–0.600 at 650 nm. The reaction was stopped with 100 µl stop solution (2N H2SO4) per well and the final absorbance was read at 450 nm.

OD readings were converted to PP values (percentage of positive control serum) using the following equation:

$$\%PP = [(\text{Mean OD of test sample} - \text{Mean OD of negative control}) / (\text{Mean OD of positive control} - \text{Mean OD of negative control})] \times 100$$

5. CHAPTER 5: RESULTS

In total 66 buffalo immobilisations took place between 15 April and 15 November, and 65 blood samples were collected from these animals for analyses (Table 1). These samples were collected from 25 individual buffalo.

A total of 15 roan immobilisations took place between 14 March and 24 October and 15 blood samples were collected from these animals for analyses (Table 2). These samples were collected from 9 inidvidual roan.

All of the above animals were vaccinated with RVF Clone 13 vaccine the first time they were immobilised, according to the recommended vaccine protocol for cattle.

5.1. Haematology and Biochemistry

Samples for both species were collected as outlined above and randomly selected for submission to IDEXX laboratories South Africa in order to detect any obvious health abnormalities that may affect the response to vaccination. Since accurate reference values are not known for these wildlife species, this data was used as a broad screening process to highlight any gross or obvious abnormalities which may reflect health concerns, and which could affect the outcome of vaccination.

Taking the above into account, this data and related comments are reported in Appendix 1 as raw data. Detailed analysis and interpretation of these results is beyond the scope of this study.

5.2 Buffalo Serum Neutralization Test and ELISA

All buffalo (23 animals; animal number 6 was not sampled and the calf was not yet born) tested negative on initial SNT testing before vaccination, from samples collected on 15 and 16 April (Table 3).

Table 3. Results of the buffalo blood samples submitted for SNT during the study.

No.	Sex	Estimated Age	RVF SNT titre						
			Blood sample collection date (2011)						
			15and16 April	26 May	23 June	28 June	12 July	1 Sept	15 Nov
1	M	18 months	Neg					Neg	Neg
2	M	18 months	Neg					Neg	Neg
3	F	2,5 years	Neg					Neg	Neg
4	F	Adult, pregnant	Neg					Neg	Neg
5	F	18 months	Neg					Neg	Neg
6	F	12 months						Neg	Neg
7	F	Adult	Neg					Neg	Neg
8	F	Mature	Neg	1:20		Neg			
9	F	18 months	Neg	1:20		Neg			
10	M	Adult	Neg	1:10		Neg			
11	F		Neg	Neg		Neg			
12	F		Neg	1:14		Neg			
13	F	Adult	Neg	1:28	Neg			Neg	Neg
14	M	Adult	Neg	Neg		Neg			
15	F	Adult	Neg	1:10		Neg			
16	F	15 months	Neg	Neg					
17	F	Adult	Neg	1:10					
18	F	12 months	Neg	Neg			Neg		
19	F	Adult	Neg				Neg		
20	F	Adult	Neg	Neg			Neg		
21	F	Mature	Neg	1:28			1:20		
22	F	Adult	Neg					Neg	Neg
23	M	15 months	Neg					Neg	Neg
24	M	Adult	Neg	>1:320			>1:320		
25	F	Born 4/2011							Neg

Notes: M = Male; F= Female; The blocked-out cells indicate that no sample was collected. Results that are 1:14 and 1:28 are half-log dilutions.

ELISA was run on the same 23 buffalo samples collected on 14 and 15 April (Table 4). All animals tested negative for IgG except for animal 12 which tested positive (titre of 37). IgM was negative for all animals.

Table 4. Results of the buffalo blood samples submitted for RVF ELISA during the study.

No.	Sex	Est.	RVF ELISA result													
		Age	Blood sample collection date (2011)													
			15 April		26-May		23-Jun		28-Jun		12-Jul		1-Sept		15-Nov	
			IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
1	M	18 mths	Neg	Neg									Neg	Neg	Neg	Neg
2	M	18 mths	Neg	Neg									Neg	Neg	Neg	Neg
3	F	2,5 yrs	Neg	Neg									Neg	Neg	Neg	4
4	F	Ad, preg	Neg	Neg									Neg	Neg	Neg	Neg
5	F	18 mths	Neg	Neg									Neg	Neg	Neg	Neg
6	F	12 mths											Neg	Neg	Neg	Neg
7	F	Adult	Neg	Neg									Neg	Neg	Neg	Neg
8	F	Mature	Neg	Neg	6	Neg			Neg	Neg						
9	F	18 mths	Neg	Neg	Neg	Neg			Neg	Neg						
10	M	Adult	Neg	Neg	5	Neg			Neg	Neg						
11	F		Neg	Neg	Neg	Neg			Neg	Neg						
12	F		37	Neg	43	Neg			Neg	Neg						
13	F	Adult	Neg	Neg	Neg	Neg	Neg	Neg					Neg	Neg	Neg	Neg
14	M	Adult	Neg	Neg	Neg	Neg			Neg	Neg						
15	F	Adult	Neg	Neg	6	Neg			Neg	Neg						
16	F	15 mths	Neg	Neg	5	Neg										
17	F	Adult	Neg	Neg	Neg	Neg										
18	F	12 mths	Neg	Neg	Neg	Neg					Neg	Neg				
19	F	Adult	Neg	Neg							Neg	Neg				
20	F	Adult	Neg	Neg	Neg	Neg					Neg	Neg				
21	F	Mature	Neg	Neg	Neg	Neg					Neg	Neg				
22	F	Adult	Neg	Neg									Neg	Neg	Neg	Neg
23	M	15 mths	Neg	Neg									Neg	Neg	Neg	Neg
24	M	Adult	Neg	Neg	Neg	34					9	5				
25	F	Newb.													Neg	Neg

Notes: M = Male; F = Female; IgG = IgG-I-ELISA, IgM = IgM-I-Elisa. Positive values >7; suspect values 4-7. The blocked-out cells indicate that no sample was collected.

Subsequent sampling on 26 May (39 or 40 days post vaccination) of 14 animals, produced nine positive SNT titres (animals 8, 9, 10, 12, 13, 15, 17, 21, 24), varying from 1:10 to more than 1:320, indicating a reaction to vaccination. The remaining five animals tested negative (Table 3). ELISA testing on the same 14 samples (Table 4) yielded four suspect results for IgG (animals 8, 10, 15 and 16), all which were previously negative (before vaccination), and one positive, animal 12 (IgG titre of 43) which was positive from the start but now exhibited a higher titre. All were negative for IgM except for animal 24 which had a positive result (titre of 34).

Two animals sampled on 23 June were erroneously reported as one negative result for a different animal and thus this result was disregarded, even though the result was not significantly different to any other results and had no impact on the overall conclusion.

Seven samples collected on 28 June (animals 8, 9, 10, 11, 12, 14 and 15) yielded seven negative SNT results (Table 3). The same seven samples above tested by ELISA resulted in all negative results (Table 4).

Five animals were sampled on 12 July (87 or 88 days post vaccination) – animals 18, 19, 20, 21, 24. On SNT testing (Table 3) animals 18, 19 and 20 were negative. Animal 21 (1:20) and animal 24 (>1:320) were positive. ELISA testing on the same samples yielded negative results for animals 18, 19, 20 and 21. Animal 24 gave a positive IgG result (9) and a suspect IgM result (5).

On 1 September (138 or 139 days post vaccination) ten samples (animals 1-7, 13, 22 and 23) were collected and all tested negative on SNT (Table 3). ELISA testing on all of these samples gave negative results.

The final 11 samples collected on 15 November included all the animals sampled on 1 September and the calf born in the boma. All 11 samples were negative on SNT testing.
On ELISA testing animal 3 gave a suspect result (4) on IgM and was negative on IgG. All other samples were negative on both IgG and IgM.

5.3 Roan antelope Serum Neutralization and ELISA Tests

Nine roan were blood sampled on 14 March for SNT and ELISA testing, prior to vaccination (Table 5 and 6). All animals were negative on SNT and ELISA (IgG and IgM) except for animal 7 which showed a positive SNT titre.

Table 5. Results of the roan antelope blood samples submitted for SNT during the study.

No.	Sex	Estimated Age	RVF SNT titre		
			Blood sample collection date		
			14 March 2011	11 April 2011	24 Oct 2011
1	F	± 3 years	Neg		
2	F	± 3 years	Neg		
3	F	30 months	Neg	Neg	
4	F	2 years	Neg		
5	F	8-10 years	Neg	1:20	1:10
6	F	10-12 years	Neg	1:10	1:28
7	F	± 6 years	1:14		1:28
8	M	± 5 years	Neg		
9	F	Old, ±12 years	Neg	1:10	

Notes: M = Male; F= Female; The blocked-out cells indicate that no sample was collected

Table 6. Results of the roan antelope blood samples submitted for RVF ELISA during the study.

			RVF ELISA result					
No.	Sex	Estimated age	Blood sample collection date					
			14-Mar-11		11-Apr-11		24-Oct-11	
			IgG	IgM	IgG	IgM	IgG	IgM
1	F	± 3 years	Neg	Neg				
2	F	± 3 years	Neg	Neg				
3	F	30 months	Neg	Neg	Neg	Neg		
4	F	2 years	Neg	Neg				
5	F	8-10 years	Neg	Neg	Neg	Neg	Neg	Neg
6	F	10-12 years	Neg	Neg	Neg	Neg	Neg	Neg
7	F	± 6 years	Neg	Neg			Neg	Neg
8	M	± 5 years	Neg	Neg				
9	F	Old, ±12 years	Neg	Neg	Neg	Neg		

Notes: M = Male; F= Female; IgG = IgG-I-ELISA. Positive values >7; suspect values between 4 - 7. IgM = IgM-ELISA. Positive values > 6; suspect values between 4 – 6.
The blocked-out cells indicate that no sample was collected.

Four roan antelope (animals 3, 5, 6, 9) were blood sampled on 11 April (28 days post vaccination) for testing (Table 5 and 6). Animals 5, 6 and 9 gave positive titres (1:10-1:20) on SNT but all were negative on ELISA (IgG and IgM).

On 24 October (214 days post vaccination) three roan antelope (animals 5, 6 and 7) were blood sampled for testing. All three showed a positive SNT titre (1:10-1:28) (Table 5) and all were ELISA negative (IgG and IgM) (Table 6).

6. CHAPTER 6: DISCUSSION

6.1 Biochemistry and haematology

The buffalo haematology and biochemistry results indicated no obvious herd or general abnormalities in these animals. The few "abnormalities" that were noted may have been due to sample quality due to the time from samples collection to processing and transport to the laboratory, and/or incorrect reference ranges. Overall, considering the laboratory specialist's comments and the animals' clinical appearance, and looking at the SNT and ELISA results, there was no evidence that any of these changes affected the vaccine reaction.

6.1.2 Roan antelope

The roan antelope haematology and biochemistry results indicated no obvious herd or general abnormalities in this population. The few "abnormalities" that were noted may have been due to sample quality due the time samples were out the field, processed and couriered to the laboratory, and incorrect reference ranges for some enzymes, notably alamine aminotransferase (ALT) and gamma-glutamyl transferase (GGT). Of more significance was the fact that a *Theileria* spp. and an *Anaplasma* spp. were evident on blood smear evaluation. All animals were infected with either one or both haemoparasites. The parasites were not typed since that was outside the scope of this research. No animals exhibited any clinical signs of disease related to these parasites, or other diseases. It is interesting to note that the two animals with the highest parasitaemias (animals 4 and 9), also had the lowest haematocrit values, although they were still not considered anaemic (Appendix 1). On repeat sampling, it was interesting to note that animal number 9 exhibited an increased *Theileria* parasitaemia on blood smear evaluation, but a decreased *Anaplasma* parasitaemia. However, this animal's haematocrit (packed cell volume as a %) increased from 22 to 33. Unfortunately, neither of these animals was sampled again in October for comparison.

In conclusion, there was no evidence that any of the haematological or biochemistry changes affected the vaccine reaction.

6.2 Adverse effects of vaccination

In sheep (Dungu, *et al*., 2010) and calves (Von Teichman, *et al*., 2011) RVF Clone 13 vaccine has been shown to be safe and effective in providing protection against virulent RVFV challenge, without any side effects. In the absence of vaccine research and associated data in wildlife, we assumed the results would be similar in wild ruminants.

No adverse effects were observed in this trial and in addition the vaccine seemed to have no ill effect on a pregnant buffalo cow, or her calf, despite the cow being vaccinated in late pregnancy. The calf was part of the group sampled and tested on 15 November and was born in the boma on 20 April (4-5 days after vaccination of her mother). Thus, the vaccine appeared to have no effect on pregnancy at this very late stage, as expected.

Most teratogenic effects are as a result of vaccination during the first trimester of pregnancy, but in addition Clone 13 has been shown to have no such side effects in sheep (Dungu *et al*., 2010). However, in contrast to this a more recent study suggests that Clone 13 virus is able to spread to the foetus, resulting in malformations and stillbirths, and thus should still be used with caution in pregnant animals, especially during the first trimester (Makoschey, *et al*., 2016).

6.3 ELISA and SNT

6.3.1 Buffalo

As expected, all animals tested negative on SNT at their initial testing on 14 and 15 April. The IgG ELISA test for animal 12 tested positive (titre = 37). This suggests previous exposure to RVF although these animals were assumed to be negative. Specific IgG antibodies, which appeared from 8 days after infection may persist for several years (Paweska, *et al*., 2005b). This finding is not uncommon, as has been shown in other studies (Lo, *et al*., 2015), and in particular one study on buffalo (Paweska *et al*., 2008). IgG-based ELISAs cannot distinguish between past and current infection unless paired serum samples are analysed (acute and convalescent) and a four-fold increase in antibody titre observed (Mansfield *et al*., 2015). The detection of IgM would suggest a current or recent infection. Since the IgM and SNT results were negative, it is likely that this animal was not infected with RVFV at the time of sampling, but either had been exposed to the virus previously or was simply a false positive.

Nine of the 14 samples collected on 26 May (39 or 40 days post vaccination) gave a positive SNT result (animals 8, 9, 10, 12, 13, 15, 17, 21, 24). Titres varied from 1:10 to more than 1:320, indicating a humoral response of some degree to vaccination. The remaining 5 animals tested negative.

ELISA testing on the same 14 samples yielded 4 suspect results for IgG (animals 8, 10, 15 and 16), all which were previously negative (before vaccination), and one positive, animal 12 (IgG titre of 43) which was positive from the start but now exhibited a higher titre. All were negative for IgM except for animal 24 which had a positive result (titre of 34). The ELISA results suggest a weak response to vaccination, with the exception of animal 24 which showed a strong response.

Based on the 28 June SNT sample results, animals 8, 9, 10, 12 and 15 which were all positive on previous sampling (26 May) were now negative. This is not consistent with findings in domestic species where positive titres persisted for longer (Von Teichman, *et al*., 2011; Lo, *et al*., 2015; Njenga *et al*. 2015), even though Njenga *et al*. (2015) did illustrate that there are definite species differences in vaccine reaction in domestic ruminants. This indicates a very poor vaccine/immune response with titres dropping to zero within 74 days post vaccination. Animals 11 and 14 were negative on both tests thus exhibiting no reaction to vaccination. Animals 9, 11 and 14 were negative on previous testing for IgG and IgM, thus their status remains unchanged. Animals 8, 10 and 15 yielded suspect results for IgG (IgM negative) on 26 May (41 or 42 days post vaccination), but were all negative (IgG and IgM) on this test. Animal 12 was positive for IgG on initial testing (IgM negative) before being vaccinated, and again positive (IgG; IgM negative) on 26 May (40 or 41 days post vaccination) with a slightly higher titre, but now negative on IgG and IgM. Since this animal was positive before vaccination no definite deductions can be made with regard to vaccine response, but as discussed above the higher titre on 26 May suggests a vaccine response. On this test a negative ELISA and SNT result, 73/74 days post vaccination, suggests that even though it appears this animal did respond to vaccination, the response was poor, as for the other animals in this test group.

Five samples collected on 12 July gave three negative SNT results. For animals 18 and 20 this is consistent with previous tests on 14/15 April and 26 May samples. This suggests that these

animals never reacted to vaccination at all, or that the response was very short lived (less than 41 or 42 days). Animal 19 was also negative and since this was the first test post vaccination (88 or 89 days) the result suggests that this animal also never reacted to vaccination, or if it did, that the positive reaction was very short lived. Animal 21 had a positive titre when tested 41 or 42 days post vaccination (26 May sampling) and was again positive on this test, even though it exhibited a 28% decrease in titre. Assuming this is a true decrease and not a testing variation, this suggests a response to vaccination with waning titres, which is quite consistent with findings in domestic species (Njenga *et al*., 2015). Animal 24 showed a high titre on 26 May testing and maintained this, suggesting a good longer lasting vaccine response.

ELISA testing of the same 5 samples produced four (animals 18, 19, 20, 21) negative results (IgG and IgM) which is consistent with their previous test results from April and May (Table 4). The results for animals 18, 19 and 20 concur with the SNT results and indicate no response to vaccination in these three animals. Animal 21 showed a positive SNT titre and so is assumed to have shown some reaction to vaccination. Animal 24 was positive for IgG (9) after being negative on a previous test result from 26 May. This animal also produced a suspect IgM result (5) after being negative prior to vaccination and positive on the 26 May test. Taking all these results into consideration it seems that this animal showed a good humoral response to vaccination.

Ten animals were sampled and tested on 1 September. This was the first test for nine of the animals (animals 1-7, 22 and 23) post vaccination, and all 9 gave a negative SNT result, the same as their first test (with the exception of animal 6 which was not tested previously). This indicates either no or a short-lived response to vaccination. Animal 13 was tested previously and showed a positive titre on a sample collected on 26 May (41 or 42 days post vaccination), a negative result on a sample collected on 23 June (69 or 70 days post vaccination) and a negative result on the 1 September sample. This suggests that there was vaccine reaction but that the immune response was very short lived and had already waned by 70 days post vaccination.

The same ten samples all tested negative on ELISA for both IgG and IgM. This was the first test post initial testing for animals 1-5, 7, 22 and 23. These animals were also negative on initial testing and these results concur with the SNT results. This was the first time animal 6 was tested and the negative result concurs with the SNT result for this animal. These results indicate

either no or a short-lived response to vaccination. Animal number 13 was tested previously on 26 May (41 or 42 days post vaccination) and 23 June (69 or 70 days post vaccination). All results were negative. This result does not concur with the SNT results for this animal which suggest a vaccine humeral response.

Eleven samples were submitted for testing on 15 November. All tested negative on SNT. Ten of these animals were the same as those samples collected on the 1st of September when they also tested negative (Table 3), thus the same comments hold true for these results. The additional sample was for animal 25, a calf born in the boma on 20 April (4-5 days after vaccination of her mother). The calf appeared healthy and was born normally. The fact that the calf exhibited a negative SNT result is also no surprise since the period from the dam being vaccinated to the calf being born was very short, not allowing any time for the dam to develop any kind of immunity which could be transferred to the calf. The calf was not vaccinated at any stage.

The same 11 samples were submitted for ELISA testing. Ten of the samples (animals 1, 2, 4, 5, 6, 7, 13, 22 and 23) were negative for both IgG and IgM. This is consistent with their previous test on 1 September thus the same comments hold true for these results. Animal number 3 was negative for IgG but yielded a suspect result for IgM whereas on the 1 September sample it tested negative for both. This, together with a negative SNT, suggests this animal is not showing a vaccine response. Animal number 25, the calf, yielded both a negative IgG and IgM ELISA result, as expected, and as per the comments in the SNT discussion above.

Taking the above discussion and results from Tables 3 and 4 into consideration, only nine buffalo had a positive SNT test result while only 2 showed a positive ELISA result, in all the tests conducted. These nine animals showed a humoral immune response post vaccination similar to what we would expect to find in domestic cattle.

Fifteen buffalo showed no humoral immune response post vaccination, or only a very short-lived response which was not confirmed by testing. Based on multiple trials with Clone 13 vaccine in domestic ruminants, one would expect most buffalo to show at least some level of humoral response post vaccination, and for titres to last longer than they did. As commented above this may be due to species differences as is seen in some domestic ruminant species too (Njenga *et al*., 2015).

Positive titres were also lower than those elicited in domestic ruminant trials with Clone 13 vaccine. We know from domestic animal trials that even animals with low titres exhibited resistance to disease challenge and so not too much emphasis can be placed on the level of the titre (Dungu *et al*., 2010) in view of the fact that the buffalo were not subjected to disease challenge.

Overall it is thus reasonable to say that the response to vaccination with Clone 13 RVF vaccine in buffalo was unpredictable, and only approximately 37.5% of animals showed any humoral response to vaccination. This is not consistent with previous research in domestic ruminant species.

The haematology and biochemistry results indicated that overall the buffalo were healthy and thus there is no obvious health reason for them not to respond to the vaccine adequately.

It may be that RVFV antibodies in buffalo may not last for as long as in domestic species, but this has not been confirmed by any previous research, nor can it be confirmed by this research.

6.3.2 Roan antelope

Based on results from initial sampling of 9 animals on 14 March, only animal 7 exhibited a positive SNT titre (1:14). All other SNT and ELISA results were negative, as expected. The positive titre indicates prior exposure to RVF although the owner of the animals assured me they had been on the property for a long time, it had been a wildlife only area for many years and there had been no local recorded cases of RVF for many years. Thus the fact that it exhibited a positive titre contributes to the theory of low level of viremia in certain animals during inter-epidemic periods, resulting in inter-epidemic transmission of RVFV, such as has been shown to occur in buffalo (LaBeaud, *et al.*, 2011). The detection of IgM would suggest a current or recent infection. Since IgG and IgM results were negative, one can assume there was no current RVFV infection in this animal, but that it had been exposed to the virus previously.

Animals 3, 5, 6, 9 were blood sampled on 11 April (28 days post vaccination) for testing. Animals 5, 6 and 9 gave positive titres (1:10-1:20) on SNT but all were negative on ELISA (IgG and IgM). Despite the discrepancy in the test these results suggest that these three animals

showed a humoral immune response post vaccination. Animal 3 was negative on all tests indicating that it failed to mount a humoral immune response post vaccination, or if it did, that this lasted less than 28 days. Either way this is a poor response to vaccination.

The three roan antelope (animals 5, 6 and 7) blood sampled on 24 October for testing all gave a positive SNT titre (1:10-1:28) and all were ELISA negative (IgG and IgM). Animal 5 showed a SNT titre of 1:20 on 11 April which had reduced to 1:10 by 24 October, while animal 6 showed a SNT titre of 1:10 on 11 April and this increased to 1:28 by 24 October. Both of these animals can be considered as showing a positive lasting humoral immune response in response to vaccination, based on these findings. Despite animal 7 having a positive SNT result prior to vaccination, it showed an increase in titre from 1:14 to 1:28. In the absence of obvious disease challenge, it can be assumed that this increase in titre is in response to vaccination.

Four out of five roan antelope that were sampled post vaccination gave a positive SNT result, but were all negative on ELISA (IgG and IgM). SNT and ELISA tests show a high correlation although it is known that this is not 100% (Njenga *et al*. 2015). These inconsistent results may be a result of species differences (Njenga *et al*., 2015), but based on the SNT results it would appear that these 4 animals showed a humoral immune response post vaccination.

Animal 3 either failed to mount a humoral immune response post vaccination, or if it did, the response lasted less than 28 days. Either way this is a poor response to vaccination based on multiple trials with Clone 13 vaccine in domestic ruminants.

The haematology and biochemistry results indicated that overall the roan were healthy and thus there is no obvious health reason for them not to respond to the vaccine adequately. This included animal 3. The roan were never in a captive facility and thus should have experienced limited stress during this trial, except for during relocation. Thus there is no clear explanation why this one individual responded differently to vaccination.

Although the positive titres were lower than those elicited in domestic ruminant trials with Clone 13 vaccine, we know from domestic animal trials that even animals with low titres exhibited resistance to disease challenge (Dungu *et al*., 2010). Thus not too much emphasis can be placed on the level of the titre in view of the fact that the roan antelope were not subjected to disease challenge.

In the roan antelope, the response to vaccination appeared to be long lasting, based on the last SNT test results 214 days post vaccination

Although this is a small number of animals and no conclusive deductions can thus be made, it appears that the roan antelope showed a good immune response to vaccination (80% tested post vaccination showed positive SNT titres), based on animals tested (N = 5). However, there is evidence in at least one animal that this response was already declining from just over 7 months post vaccination, based on reducing SNT titres. This is much sooner than expected when compared to studies done on domestic ruminants (Njenga *et al*., 2015).

6.4 General

The roan antelope showed a better humoral response to vaccination than the buffalo.

One of the possible reasons for this poor response in both of these species could be a defective vaccine, although although this is unlikely considering some individuals did respond to vaccination. Since the efficacy of the vaccine was not tested this cannot be confirmed, but the vaccine was sent directly from the manufacturer on ice and thus there is no reason to doubt the integrity of the vaccine. The vaccine was also maintained on ice in the field. Clone 13 vaccine has been proven to be both safe and effective in domestic ruminant trials (Von Teichman, *et al*., 2011, Hunter, 2001), and all vaccinated animals elicited immune responses that are protective against challenge with virulent RVFV.

The vaccination dose could be low, but again this is unlikely considering research done in domestic cattle (Von Teichman, *et al*. 2011; Njenga, *et al*., 2015). There could however be species-specific factors which necessitate a higher dose in order to elicit a better humoral response, as eluded to by some of the results in the latter study. In addition, booster vaccines may be required in order to elicit a longer lasting immune response.

The stress that the animals had been exposed to may play a role. Despite animals appearing to be in good health, they were all caught from the wild and placed in a captive situation during this trial and would have experienced very high levels of stress. This would have compromised their immune systems, perhaps more in some individuals than others, which also offers a

possible explanation as to the varied response to vaccination between individuals. This is a complex topic, and was not investigated at all in this study.

A possible but unlikely factor may be that the SNT and ELISA results are inconsistent. This again seems unlikely since all samples were submitted to the same testing procedures and some showed positive results/results as expected, while others did not. It needs to be emphasized that the SNT and ELISA tests were validated for domestic animals and not for wildlife, so it can be expected that species differences occur. In an animal with a weak immune response, the SNT may not even be sensitive enough to identify low concentrations of antibodies for example.

6.5 Conclusions

In conclusion, it would appear that there are significant species differences in immune response post vaccination with Clone 13 vaccine. Wildlife vaccine protocols and expected outcomes thus cannot reliably be based on those of domestic ruminants.

It would appear that there is no obvious health risk in vaccinating buffalo or roan antelope with Clone 13 vaccine and that some immune response is induced, albeit inconsistent. It should however be noted that safety in different stages of pregnancy, especially first trimester, has not been established by this trial and caution should be exercised in this regard.

Based on the roan results, vaccination with RVF Clone 13 vaccine can be considered a potential option for protecting this species and other valuable wildlife species against the risk of RVF disease, but requires further investigation, and the results may vary from species to species.

Based on the poor buffalo results, it would appear that vaccination with RVF Clone 13 vaccine does not induce an adequate humeral immune response in this species when following the recommended protocol for domestic cattle.

Ultimately a trial which involves challenging vaccinated animals with virulent RVFV will be the only reliable way to test for an adequate immune response elicited by vaccination. Current value of roan antelope prevents such a trial from taking place. Perhaps such a trial would be possible in buffalo using animals that are not disease free, and hence less valuable, but then the issue of other diseases such as tuberculosis interfering in the vaccine/immune response would

have to be considered. Disease-free buffalo, like roan, are very valuable and thus also unlikely to be available for such a trial.

Validation of the SNT and ELISA tests for valuable wildlife species will also need to be undertaken for further meaningful research outcomes.

Future research into vaccinating valuable wildlife species against RVF should consider the findings of this study as a base on which to build a more targeted research model, as far as possible considering the value and practicalities of such a trial in wildlife.

7. REFERENCES

Albarino C.G., Bird B.H. and Nichol S.T., 2007. A shared transcription termination signal on negative and ambisense RNA genome segments of Rift Valley fever, sandfly fever Sicilian, and Toscan viruses. *Journal of Virology*, 81:5246-5256.

Alexander R.A., 1951. Rift Valley fever in the Union. *Journal of the South African Veterinary Medical Association*, 22:105-109.

Alexander R.A., 1957. Discussion on both Rift Valley fever (RVF) and Wesselsbron virus disease (WVD). Proceedings of the 4th Meeting of the Inter-African Advisory Committee on Epizootic Diseases, Dakar, Senegal, 1-11 May 1957:29.

Anderson G.W. and Peters C.J., 1988. Viral determinants of virulence for Rift Valley fever (RVF) in rats. *Microbial Pathogenesis*, 5:241-250.

Anderson G.W. Jr, Saluzzo J.F., Ksiazek T.G., Smith J.F., Ennis W. and Thureen D., 1989. Comparison of in vitro and in vivo systems for propagation of Rift Valley fever virus from clinical specimens. *Research Virology*, 140:129-138.

Anderson G.W. J. and Smith, J.F., 1987. Immunoelectron microscopy of Rift Valley fever viral morphogenesis in primary rat hepatocytes. *Virology*, 161:91-100.

Archer B.N., Weyer J., Paweska J., Nkosi D., Leman P., Tint K.S. and Blumberg L., 2011. Outbreak of Rift Valley fever affecting veterinarians and farmers in South Africa, 2008. *South African Medical Journal*, 101(4): 263-266.

Assad F., Davies F.G and Eddy G.A., 1983. The use of veterinary vaccines for prevention and control of Rift Valley fever. *Bulletin of the World Health Organisation*, 61:261-268.

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

Barnard B.J.H, 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.

Barnard B.J.H. and Botha M.J., 1977. An inactivated Rift Valley fever vaccine. *Journal of the South African Veterinary Association*, 48:45-48.

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

Beechler B. R., Bengis R., Swanepoel R., Paweska J. T., Kemp A., van Vuren P. J., Joubert J., Ezenwa V. O. and 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. doi:10.1111/tbed.12197.

Besselaar T.G. and Blackburn N.K., 1991. Topological mapping of antigenic sites on the Rift Valley fever virus envelope glycoproteins using monoclonal antibodies. *Archives of Virology*, 121, 111-124.

Bird B.H., Baweic D.A., Ksiazek T.G., Shoemaker T.R. and Nichol S.T, 2007. Highly sensitive and broadly reactive quantitative RT-PCR assay for high throughput detection of Rift valet fever virus. *Journal of Clinical Microbiology*, 45:3505-3513.

Bird B.H., Githinji J.W.K., Macharia J.M., Kasiiti J.L., Muriithi R.M., Gacheru S.G., Musaa J.O., Towner J.S., Reeder S.A., Oliver J.B., Stevens T.L., Erickson B.R., Morgan L.T., Khristova M.L., Hartman A.L., Comer J.A., Rollin P.E., Ksiazek T.G. and Nichol S.T., 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.

Bird B.H., Khristova M.L., Rollin P.E., Ksiazek T.G. and Nichol S.T., 2007. Complete genome analysis of 33 ecologically and biologically diverse Rift Valley Fever virus strains reveals

widespread virus movement and low genetic diversity due to recent common ancestry. *Journal of Virology*, 81:2805-2816.

Burroughs R., Hofmeyr M., Morkel P., Kock M.D., Kock R. and Meltzer D., 2012. Chemical immobilization – individual species requirements. In: MD Kock and R Burroughs (Eds.), Chemical and physical restraint of wild animals, a training and field manual for African species, Second edition, IWVS (Africa), Greyton, South Africa, 148-151; 183-185.

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

Coetzer J.A.W., 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.

Coetzer J.A.W., 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.

Coetzer J.A.W. and Barnard B.J.H., 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.

Collett, M.S., 1986, Messenger RNA of the M segment RNA of Rift Valley fever virus. *Virology*, 151(1), 151-156.

Data sheet, Inactivated Rift Valley fever vaccine for cattle, sheep and goats, Onderstepoort Biological Products, Private Bag X07, Onderstepoort, 0110, South Africa.

Data sheet, Live Rift Valley fever vaccine for cattle, sheep and goats, Onderstepoort Biological Products, Private Bag X07, Onderstepoort, 0110, South Africa.

Data sheet, Rift Valley fever Clone 13 vaccine for cattle, sheep and goats, Onderstepoort Biological Products, Private Bag X07, Onderstepoort, 0110, South Africa.

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

Davies F.G., 1975. Observations on the epidemiology of Rift Valley fever in Kenya. *Journal of Hygiene*, 75:219-230.

Davies F.G., 1981. Rift Valley fever in Kenya. *Proceedings of the 49th General Session of the Office International de Epizooties*, Paris, 25-30 May.

Davies F.G., and Addy P.A.K., 1979. Rift Valley fever. A survey for antibody to the virus in bird species commonly found in situations considered to be enzootic. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 73:584-585.

Davies, F.G. and Karstad L., 1981. Experimental infection of the African buffalo with the virus of Rift Valley fever. *Tropical Animal Health and Production*, 13:185-188.

Davies F.G., Linthicum K.J., and James A.D., 1985. Rainfall and epizootic Rift Valley fever. *Bulletin of the World Health Organization*, 63:941-943.

Davies F.G. and Martin V., 2006. Recognizing Rift Valley Fever. *Veterinaria Italiana* 42(1): 7-53.

Dautu G., Sindato C., Mweene A.S., Samui K.L., Roy P., Noad, R. *et al.*, 2012. Rift Valley fever: Real or perceived threat for Zambia? *Onderstepoort Journal of Veterinary Research*, 79(2), 94-99.

Directorate of Animal Health, 2012. National Animal Disease Database of the Directorate of Animal Health 1992 to 2012. Department of Agriculture, Forestry and Fisheries, Pretoria.

Drosten C., Gottig S., Schilling S., Asper M., Panning M., Schmidtz H., Gunther S., 2002. Rapid detection and quantification of RNA of Ebola and Marburg viruses, lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time revers transcription-PCR. *Journal of Clinical Microbiology*, 40:2323-2330

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

Easterday B.C., 1965. Rift Valley fever. *Advances in Veterinary Science*, 10:65-127.

Easterday B.C., Mc G.M., Rooney J.R. and Murphy L.C., 1962. The pathogenesis of Rift Valley fever in lambs. *American Journal of Veterinary Research*, 23:470–479.

Eisa M., Obeid H.M.S. and El Saei A.S.A., 1977. Rift Valley fever in the Sudan. I. Results of field investigations of the first epizootic in Kosti District, 1973. *Bulletin of Animal Health and Production in Africa*, 24:343-347

Ellis D.S., Shirodaria P.V., Flemming E. and Simpson D.I.H., 1988. Morphology and development of Rift Valley fever virus in Vero cell cultures. *Journal of Medical Virology*, 24:161-174

El Mamy A.B.O., Baba, M.O., Barry Y., Isselmou K., Dia M.L., Hampate B., Diallo M.Y., El Kory M.O.B, Diop M., Lo M.M., Thiongane Y., Bengoumi M., Puech L., Plee L., Claes F., De La Rocque S. and Doumbia B., 2011. Unexpected Rift Valley fever outbreak, Northern Mauritania. *Emerging Infectious Diseases*, 17(10): 1894–1896. http://doi.org/10.3201/eid1710.110397.

Evans A., Gakuya F., Paweska J.T., Rostal M., Akoolo L., Van Vuren P.J., Manyibe T., Macharia J.M., Ksiazek T.G., Feikin D.R., Breiman R.F. and Njenga M.K., 2008. Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife. *Epidemiology and Infection*, 136:1261-1269.

Fafetine J. M., Coetzee P., Mubemba B., Nhambirre O., Neves L., Coetzer, J. and Venter, E. H., 2016. Rift Valley fever outbreak in livestock, Mozambique, 2014. *Emerging Infectious Diseases*, 22(12), 2165-2167.

Fagbo S., Coetzer J.A.W. and 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(1), 01-07.

Formenty P., Domenech J. and Zeller H.G., 1992. Serological survey of Rift Valley fever in sheep on the Ivory Coast. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, 45:221-226.

Freed I., 1951. Rift Valley fever in man complicated by retinal changes and loss of vision. *South African Medical Journal*, 25:930-932.

Freiberg, A.N., Sherman, M.B., Morais, M.C., Holbrook, M.R. and Watowich, S.J., 2008. Three-dimensional organization of Rift Valley fever virus revealed by cryoelectron tomography. *Journal of Virology*, 82(21), 10341-10348.

Gad A.M., Feinsod F.M., Allan I.H., Eisa M., Hassan A.N., Soliman B.A., 1986. A possible route for the introduction of Rift Valley fever virus into Egypt during 1977. *Journal of Tropical Medicine and Hygiene*, 89:233-236.

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

Gear, J.H.S., 1977. Haemorrhagic fevers of Africa: An account of two recent outbreaks. *Journal of the South African Veterinary Association*, 48:5-8.

Gear J.H.S., De Meillon B., Le Roux A.F., Kofski R., Rose-Innes R., Steyn J.J., *et al*., 1955. Rift Valley fever in South Africa: study of the 1953 outbreak in the Orange Free State, with special reference to the vectors and possible reservoir hosts. *South African Medical Journal*, 29:514-518.

Gerdes G.H., 2004. In: Office International des Epizooties (ED.), Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds, bees), Chapter 2.1.8., Paris, 2004, pp185-194.

Gerrard, S.R., Bird, B.H., Albariño, C.G. and Nichol, S.T., 2007, The NSm proteins of Rift Valley fever virus are dispensable for maturation, replication and infection, *Virology*, 359(2) 459-465.

Groenewald B. and York R., 2013. An economic outlook: The Wildlife Industry. *Game and Hunt*, 19(7): 99-104.

Gunther S., 2002. Rapid detection and quantification of RNA of Ebola and Marburg viruses, lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time revers transcription-PCR. *Journal of Clinical Microbiology*, 40:2323-2330.

Himeidan Y.E., Kweka E.J., Mahgoub M.M., El Rayah EA. and Ouma J.O., 2014. Recent outbreaks of Rift Valley fever in East Africa and the Middle East. *Frontiers in Public Health*, 2:169, 11.

Hunter B., 2001. Investigation of C13 RVF mutant as a vaccine strain. *Proceedings of 5th International sheep veterinary congress*; pp. 21–25.

Hoogstraal H., Meegan J.M., Khalil G.M. and Adham F.K., 1979. Rift Valley fever epizootic in Egypt 1977-1978. II. Ecological and entomological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 73:624-629.

Hubbard K.A., Baskerville A. and Stephenson J.R., 1991. Ability of a mutagenized virus variant to protect young lambs from Rift Valley fever. *American Journal of Veterinary Research*, 52:50-55.

Hunter P., Erasmus B.J. and Vorster J.H, 2001. Teratogenicity of a mutagenized Rift Valley fever virus (MVP12) in sheep. *Onderstepoort Journal of Veterinary Research*, 69:95-98.

Hussein N.A., Chizyuka R.Z., Ksiazek T.G., Scott R.McN. and Boulos B.A.M., 1987. Epizootic of Rift Valley fever in Zambia, 1985. *The Veterinary Record*, 121:111.

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

Ikegami, T. and Makino, S., 2011. The pathogenesis of Rift Valley fever. *Viruses*, 3(5):493–519.

Ikegami, T., Won, S., Peters, C.J. and Makino, S., 2007. Characterization of Rift Valley fever virus transcriptional terminations. *Journal of Virology*, 81(16), 8421-8438.

Jansen van Vuren, P., Potgieter, A.C., Paweska, J.T. and 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*, 140:06-114.

Joubert J.D.S., Ferguson A.L. and Gear J., 1951. Rift Valley fever in South Africa. II. The occurrence of human cases in the Orange Free State, the North-western Cape Province, the Western and Southern Transvaal. A. Epidemiological and clinical findings. *South African Medical Journal*, 25:890-891.

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

Kakach, L.T., Wasmoen, T.L. and Collett, M.S., 1988, Rift valley fever virus M segment: Use of recombinant vaccinia viruses to study Phlebovirus gene expression. *Journal of Virology*, 62(3), 826-833.

Kaschula V.R., 1953. The propogation 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.

Knauert F.K., Meegan J.M., Jouan A., Ksiazek T.G., Leguenno B. and Sarthou J.L., 1989. Assessment of an rDNA probe filter hybridization assay for detection of Rift Valley fever virus RNA in human serum samples from the Mauritian epidemic. *Research in Virology*, 140:47-57.

Kokernot R.H, Heymann C.S., Muspratt J. and Wolstenholme B., 1957. Isolation of Bunyamwere and Rift Valley fever viruses from mosquitoes. *South African Journal of Medical Sciences*, 22:71-80.

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 and Parasitology*, 55:73-85.

Kokernot R.H., Smithburn K.C. and Weinbren M.P., 1956. Neutralizing antibodies to arthropod-borne viruses in human beings and animals in the Union of South Africa. *Journal of Immunology*, 77:313-323.

Ksiazek T.G., Jouan A., Meegan J.M., Leguenno B., Wilson M.L. and Peters C.J., 1989. Rift Valley fever among domestic animals in the recent West African outbreak. *Research in Virology*, 140:67-77.

LaBeaud A.D., Cross P.C., Getz W.M., Glinka A. and King C.H., 2011. Rift Valley fever virus infection in African buffalo (Syncerus caffer) herds in rural South Africa: Evidence of interepidemic transmission. *American Journal of Tropical Medicine and Hygiene*, 84:641-646.

Le Roux C.A, Kubo T., Grobbelaar A.A., Van Vuuren P.J., Weyer J. and Nel L.H., 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.

Linthicum K.J., Davies F.G., Kairo A. and Bailey C.L., 1985. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Journal of Hygiene*, 95:197-209.

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

Logan T.M., Linthicum K.J., Wagateh J.N., Thande P.C., Kamau C.W and Roberts C.R., 1990. Pretreatment of floodwater *Aedes* habitats (dambos) in Kenya with a sustained-release formulation of methoprene. *Journal of the American Mosquito Control Association*, 6:736-738.

Makoschey B., Van Kilsdonk E., Hubers W.R., Vrijenhoek M.P., Smit M., Wichgers Schreur P.J., Kortekaas J. and Moulin V., 2016. Rift Valley fever vaccine virus Clone 13 is able to cross the ovine placental barrier associated with foetal infections, malformations, and stillbirths. *Neglected Tropical Diseases*, https://doi.org/10.1371/journal.pntd.0004550.

Mandell R.B., Koukuntla R., Mogler L.J.K., Carzoli A.K., Holbrook M.R., Martin B.K., Vahanian N., Link C.J. and Flick R., 2010. Novel suspension cell-based vaccine production systems for Rift Valley fever virus-like particles. *Journal of Virological Methods*, 169 (2): 259-268.

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

Matthee C.A. and Davis S.K., 2001. Molecular insights into the evolution of the Family Bovidae: A nuclear DNA perspective. *Molecular Biology and Evolution*, 18(7): 1220-1230.

Matumoto M., Iwasa S. and Endo M., 1950. Complement fixation reaction of Rift Valley fever virus. *Japanese Journal of Experimental Medicine*, 20:501-508.

McIntosh, B.M., 1972. Rift Valley fever. I. Vector studies in the field. *Journal of the South African Veterinary Association*, 43:391-395.

McIntosh, B.M., 1980. The epidemiology of arthropod-borne viruses in Southern Africa. D.Sc. Thesis, University of Pretoria.

McIntosh B.M., Russell D., Dos Santos I. and Gear, J.H.S., 1980. Rift Valley fever in humans in South Africa. *South African Medical Journal*, 58: 803-806.

Meegan J.M., 1981. Rift Valley fever in Egypt: An overview of the epizootics in 1977 and 1978. *Contributions to Epidemiology and Biostatistics*, 3: Rift Valley fever, 100-113.

Meegan J.M., and Bailey C.L., 1989. Rift Valley fever. In: Monath T.P. (ed), The arboviruses: Epidemiology and ecology, CRC Press, Inc, Boca Raton, Florida, 4:51-76.

Meegan J.M., Leguenno B., Ksiazek T., Jouan A., Knauert P. and Digoutte J.P., 1989. Rapid diagnosis of Rift Valley fever: a comparison of methods for the direct detection of viral antigen in human sera. *Research in Virology*, 140:59-65.

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

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

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

Mundel B. and Gear J., 1951. Rift Valley fever. The occurrence of human cases in Johannesburg. *South African Medical Journal*, 25:797-800.

Niklasson B., Grandien M., Peters C.J. and Gargan T.P.I., 1983. Detection of Rift Valley fever antigen by enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, 17:1026-1031.

Njenga M.K., Njagi L., Thumbi S.M., Kahariri S., Githinji J., Omondi E., Baden A, Murithi M., Paweska J., Ithondeka P.M., Ngeiywa K.J., Dungu B., Donadeu M. and Munyua P.M., 2015. Randomized controlled field trial to assess the immunogenicity and safety of rift valley fever Clone13 vaccine in livestock. *PLoS Neglected Tropical Diseases*, 9(3):e0003550. doi:10.1371/journal.pntd.0003550.

OIE 2008. Rift Valley fever, in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (http://www.oie.int/eng/en_index.html).

OIE 2009. Rift Valley fever, in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (http://www.oie.int/eng/en_index.html).

OIE 2010. Rift Valley Fever, in Terrestrial Manual, Chapter 2.1.14. (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.14_RVF.pdf).

OIE 2014. Rift Valley Fever, in Terrestrial Manual, Chapter 2.1.14. (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.14_RVF.pdf).

Olaleye O.D., Tomori O., Fajimi J.L. and Schmitz H., 1996. Experimental infection of three Nigerian breeds of sheep with the Zinga strain of the Rift Valley fever virus. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, 49:6-16.

Olive M.M., Goodman S.M. and Reynes JM., 2012. The role of wild mammals in the maintenance of Rift Valley fever virus. *Journal of Wildlife Diseases*, 48(2): 241–266.

Paweska, J.T., Burt, F.J., Anthony, F., Smith, S.J., Grobbelaar, A.A., Croft, J.E., Ksiazek, T.G. and Swanepoel, R., 2003a. 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(2), 103-112.

Paweska, J.T., Burt, F.J., Anthony, F., Smith, S.J., Grobbelaar, A.A., Croft, J.E., Ksiazek, T.G. and Swanepoel, R., 2003b. 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(2), 103-112.

Paweska, J.T., Burt, F.J. and 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(1-2), 173-181.

Paweska, J.T., Mortimer, E., Leman, P.A. and 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(1), 10-18.

Paweska, J.T., Jansen van Vuren, P. and 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(1-2), 119-124.

Paweska, J.T., Smith, S.J., Wright, I.M., Williams, R., Cohen, A.S., Van Dijk, A.A., Grobbelaar, A.A., Croft, J.E., Swanepoel, R. and Gerdes, G.H., 2003c. Indirect enzyme-linked immunosorbent assay for the detection of antibody against Rift Valley fever virus in domestic and wild ruminant sera. *Onderstepoort Journal of Veterinary Researc*h 70(1), 49-64.

Paweska, J.T., van Vuren, P.J., Kemp, A., Buss, P., Bengis, R.G., Gakuya, F., Breiman, R.F., Njenga, M.K. and Swanepoel, R., 2008, Recombinant nucleocapsid-based ELISA for detection of IgG antibody to Rift Valley fever virus in African buffalo. *Veterinary Microbiology* 127(1-2), 21-28.

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

Petisca J.L.N. and Serra J.J.B.L., 1971. Anatomia patoligica de algumas doencas dos animais domesticos. VI. Febre do Vale de Rift. *Veterinaria Mocambicana*, 4:69-73.

Peters C.J. and Anderson G.W., 1981. Pathogenesis of Rift Valley fever. *Contributions to Epidemiology and Biostatistics*, 3:21-41.

Peters C.J., Ennis W.H., Turell M.J. and Niklasson B., 1989. Rapid detection of Rift Valley fever antigen in the serum of infected lambs. *Research in Virology*, 140:43-46.

Peyrefitte C.N., Boubis L., Coudrier D., Bouloy M., Grandadam M., Tolou H.J. and Plumet S., 2008. Real-time reverse-transcription loop-mediated isothermal amplification for rapid detection of Rift Valley fever virus. *Journal of Clinical Microbiology*, 46:3653-3659.

Pienaar, N.J. and 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), Art. #384, 13 pages.

Pini A., Lund L.J., and Davies F.G., 1970. Detection of Rift Valley fever virus by the fluorescent antibody technique in organs of experimentally infected animals. *Research in Veterinary Science*, 11:82-85.

The Red Data Book of the Mammals of South Africa: A Conservation Assessment, Endangered Wildlife Trust, 2004.

Rice R.M., Erlick B.J., Rosato R.R., Eddy G.A. and Mohanty S.B., 1980. Biochemical characterisation of Rift Valley fever virus. *Virology*, 105:256-260.

Sall A.A., Macondo E.A., Sene O.K., Diagne M., Sylla R., Mondo M., 2002. Use of reverse transcriptase PCR in early diagnosis of Rift Valley fever. *Clinical and Diagnostic Laboratory Immunology*, 9:713-715.

Schmaljohn C.S. and Nichol S.T., 2007. Bunyaviridae. IN: Fields, BN, Knipe DM, Howley PM , Griffin, DE Fields Virology, 5th Edition. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia 1741-1789.

Scott G.R., Weddell W. and Reid D., 1956. Preliminary findings on the prevalence of Rift valley fever in Kenya cattle. *Bulletin of Epizootic Diseases of Africa*, 4:17-25.

Sellers R.F., Pedgley D.E. and Tucker M.R., 1982. Rift Valley fever, Egypt – 1977: disease spread by wind-borne insect vectors? *The Veterinary Record*, 110:73-77.

Sherman M.B., Freiberg A.N., Holbrook M.R. and Watowich, S.J., 2009. Single-particle cryo-electron microscopy of Rift Valley fever virus. *Virology*, 387(1): 11-15.

Shimshony A, and Barzilai R., 1983. Rift Valley fever. *Advances in Veterinary Science and Comparative Medicine*, 27:347-425.

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. and Nichol, S.T., 2002. Genetic analysis of viruses associated with emergence of Rift Valley fever in Saudi Arabia and Yemen, 2000-01. *Emerging Infectious Diseases*, 8(12), 1415-1420.

Sissoko D., Giry C., Gabrie P., Tarantola A., Pettinelli F., Collet L., D'Ortenzio E., Renault P. and Pierre, V., 2009. Rift Valley fever, Mayotte, 2007–2008. *Emerging Infectious Diseases*, 15(4): 568–570. http://doi.org/10.3201/eid1504.081045 .

Smithburn K.C., Haddow A.J. and Gillett J.D., 1948. Rift Valley fever: Isolation of the virus from wild mosquitoes. *British Journal of Experimental Pathology*, 29:107-121.

Smithburn K.C., Haddow A.J. and Lumsden W.H.R., 1949. Rift Valley fever: Transmission of the virus by mosquitoes. *British Journal of Experimental Pathology*, 30:35-47.

Sow A., Faye O., Ba Y., Ba H., Diallo D., Faye O., Loucoubar C., Boushab M., Barry Y., Diallo M. and Sall, A.A., 2014. Rift Valley fever outbreak, southern Mauritania, 2012. *Emerging Infectious Diseases*, 20(2), 296–299. http://doi.org/10.3201/eid2002.131000 .

Stordy R.J., 1913. Mortality among lambs. Annual Report, Department of Agriculture, British East Africa, 1912-13.

Swanepoel R., 1976. Studies on the epidemiology of Rift Valley fever. *Journal of the South African Veterinary Medical Association*, 47:93-94.

Swanepoel R., 1981. Observations on Rift Valley fever in Zimbabwe. *Contributions to Epidemiology and Biostatistics*, 3:83-91.

Swanepoel, R., 1990. National Institute for Virology, Sandringham, 2131. Unpublished observations, 1969-1990.

Swanepoel R. and Coetzer J.A.W., 2004. Rift Valley fever. In: JAW Coetzer, GR Thomson, RC Tustin (Eds.), Infectious diseases of livestock with special reference to southern Africa, Oxford University Press, Cape Town, 1037-1070.

Swanepoel R., Manning B. and Watt J.A., 1979. Fatal Rift Valley fever affecting humans in South Africa: A clinico-pathological study. *Central African Journal of Medicine*, 25:1-8.

Swanepoel R., Struthers J.K., Erasmus M.J., Shepherd S.P., and McGillivray G.M., 1986. Comparison of techniques for demonstrating antibodies to Rift Valley fever virus. *Journal of Hygiene*, 97:317-329.

Tambo E., Olalubi O.A. and Sacko M., 2016. Rift valley fever epidemic in Niger near border with Mali. *The Lancet, Infectious diseases*, 16 (12):1319–1320.

Tomori O., 1979. Clinical, virological and serological response of the West African dwarf sheep to experimental infection with different strains of Rift Valley fever virus. *Research in Veterinary Science*, 26:152-159.

Turell M.J., Faran M.E., Cornet M. and Bailey C.L., 1988. Vector competence of Senegalese Aedes fowleri (Diptera: Culicidae) for Rift Valley fever virus. *Journal of Medical Entomology*, 25:262-266.

Van der Linde N.T., 1953. A recent epidemic of Rift Valley fever in the Orange Free State. *Journal of the South African Veterinary Medical Association*, 24:145-148.

Van der Lugt J.J., Coetzer J.A. and Smit M.M., 1996. Distribution of viral antigen in tissues of new-born lambs infected with rift valley fever virus. *Onderstepoort Journal of Veterinary Research*, 63:341–347.

Van Velden D.J.J., Meyer J.D., Oliver J., Gear J.H.S. and McIntosh B., 1977. Rift Valley fever affecting humans in South Africa. *South African Medical Journal*, 51:867-871.

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

Weiss K.E., 1957. Rift Valley fever – a review. *Bulletin of Epizootic Diseases of Africa*, 5:431-458.

Weiss K.E., 1962. Studies on Rift Valley fever – passive and active immunity in lambs. *Onderstepoort Journal of Veterinary Research*, 29:3-9.

Whittle R.K., Linthicum K.J., Thande P.C., Wagati J.N., Kamau C.M. and Roberts C.R., 1993. Effect of controlled burning on survival of floodwater Aedes eggs in Kenya. *Journal of the American Mosquito Control Association*, 9:72-77.

Williams R., Ellis C.E., Smith S.J., Potgieter C.A., Wallace D., Mareledwane V.E. and Majiwa P.A.O., 2011 Validation of an IgM antibody capture ELISA based on a recombinant nucleoprotein for identification of domestic ruminants infected with Rift Valley fever virus. 2011. *Journal of Virological Methods*, 177(2)140-146.

World Health Organization 1982. Rift Valley fever: An emerging human and animal problem, in WHO offset publication 63.
(http://apps.who.int/iris/bitstream/10665/37302/1/WHO_OFFSET_63.pdf)

World Health Organization, 2007. Outbreaks of Rift Valley fever in Kenya, Somalia and United Republic of Tanzania, December 2006–April 2007. *Weekly Epidemiological Record*, 82:169–78.

Yedloutschnig R.J., Dardiri A.H. and Walker J.S., 1981. Persistence of Rift Valley fever virus in the spleen, liver and brain of sheep after experimental infection. *Contributions to Epidemiology and Biostatistics*, 3: 53-59.

8. Appendix 1: Haematology and Biochemistry Results and Discussion

A “general medical profile for production animals” was run on blood samples for biochemistry evaluation, and this included testing for sodium (Na), potassium (K), calcium, alanine aminotransferase (ALT), alkaline phosphatase (ALP), inorganic phosphate, urea, creatinine (serum), total bilirubin, total protein (serum), albumin, globulin, glucose (serum), chloride (Cl), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), creatine kinase (CK) and lactate dehydrogenase (LDH).

A full blood count was run for haematological evaluation and the parameters measured included red blood cell count, haemoglobin, haematocrit (PCV) – spun (Ht), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), white cell count (WCC) and platelet count (Thr). A differential count was also run and included segmented neutrophils (%), band neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%), basophils (%) and absolute counts of segmented neutrophils (Neu), band neutrophils, lymphocytes (Lym), monocytes (Mono), eosinophils (Eos) and basophils.

The results in the tables below highlight only biochemistry abnormalities and any parameters not listed below were considered by the IDEXX veterinary specialist to be normal. All haematology data has been listed and abnormalities indicated as the specialist comments.

Buffalo

Ten samples from 23 samples collected on 15 and 16 April were randomly selected for submission and significant results are tabled below.

Parameter	1	3	4	5	10	14	15	16	18	23
Na					149		151	154		151
K	9.80	11.8	12.9	14.0	8.30	7.50	7.90	7.30	10.0	8.00
ALT	82	107	92	120	110	125	106	111	110	125
ALP					353					
Urea	0.70	1.00	1.40		0.80	0.70	1.00	0.60		
Glucose	11.1									
AST				301						
GGT	30			28	37		41	39	32	26
LDH	1653	1967	2627	2794	1778	2032	1942	1897	2967	2819
Ht	42	29	36	38	33	48	30	40	34	32
WCC	11.4	9.03	9.95	9.94	13.91	6.66	7.64	9.71	11.26	8.80
Thr	134	235	207	384	144	162	254	191	261	174
Neu	2.74	1.81	3.08	1.69	0.97	1.93	3.21	1.36	2.03	1.14
Lym	8.22	6.68	6.37	8.25	12.24	3.93	2.83	8.06	8.90	6.78
Mono	0.23	0.18	0.20	0.00	0.14	0.13	0.99	0.10	0.11	0.09
Eos	0.23	0.36	0.30	0.00	0.56	0.67	0.69	0.19	0.23	0.79
Theileria %	0.008	0.02	0.02	0.01	0.008	0.003	0.001	0.02	0.06	0.09

Key:

1 = buffalo number 1, 3 = buffalo number 3, etc.

Yellow highlighted data considered an invalid result

Red highlighted data are clinically significant

Lavender highlighted are of possible clinical significance

Considering the results tabled above and the comments provided by Dr Fred Reyers (BVSc (Hons) MMedVet (KLD)), a clinical pathology specialist consulting for IDEXX Laboratories

South Africa, when the samples were analysed, the following interpretation of results was provided:

The potassium values are invalid and the most likely reason for this is that the cells (especially red cells) were left in contact with the serum for too long and/or that transit temperatures were too high. This also casts some doubt over other data such as ALT and LDH which may also "leak" from cells.

It is evident from these data that the given reference range for ALT (especially) and GGT (to a degree) are not appropriate and need to be adjusted.

There was considerable clotting evident in some of the EDTA samples and consequently some of the lower platelet counts are spurious. None of the counts, however, suggest clinically significant pathology.

The lymphocyte counts (in 8 of the ten animals), by any standard (local or ISIS reference ranges), are surprisingly high (confirmed by smear examination) and very probably attest to a significant antigenic stimulation. However, only one (with the highest count - see below) reveals additional evidence of possible pathology.

The data from one animal, buffalo number 10, suggest that this animal is very different from the others and may actually have significant clinically inapparent (assuming that these animals were clinically normal) disease.

The presence of piroplasms of Theileria spp. was noted on blood smear evaluation (possibly *T. buffeli*). It is possible, though unlikely, that these parasites represent *Theileria parva*. Only PCR could resolve the finite identification.

Considering the above comments, it would appear that all animals were clinically normal at the time of capture and vaccination, except for buffalo number 10 which potentially had some underlying disease process going on. However, looking at the SNT and ELISA results below this animal did not react markedly differently when compared to other animals in the group and so it can be assumed that whatever the process that was going on, it did not affect the response to vaccination significantly.

The presence of *Theileria* spp. piroplasms is not abnormal and all animals tested negative for Theileria parva as part of the State Veterinary Services disease testing protocol for buffalo at the time. This is a Reverse Line Blot Hybridisation Test run according to the SOP at the Agricultural Research Council's Onderstepoort Veterinary Research Institute. The animals also showed no other clinical signs of *Theileria*-related disease during their extended stay in captivity. It is thus reasonable to assume this factor would have had no significant effect on the animals' response to vaccination.

One sample from 2 samples collected on 23 June was randomly selected for submission and significant results are tabled below.

Parameter	Buffalo 16
Na	
K	93
ALT	
ALP	
Urea	
Glucose	
AST	307
GGT	
LDH	1558
Ht	51
WCC	5.44
Thr	125
Neu	3.37
Lym	1.69
Mono	0.38
Eos	0.00
Theileria %	0.07

Considering the results tabled above for buffalo number 16, the following comments were provided by Dr Fred Reyers:

The elevation of serum LDH activity and the associated elevation of AST, is probably incidental and may represent simple bruising.

It is evident from these data that the given reference range for ALT (especially) and GGT (to a degree) are not appropriate and need to be adjusted.

The presence of piroplasms of *Theileria* spp was noted on blood smear evaluation (possibly T buffeli). It is possible, though unlikely, that these parasites represent *Theileria parva*. Only PCR could resolve the finite identification. The absence of evidence of lymphoid cell transformation and the still relatively low parasitaemia would be consistent with this being an incidental finding.

Considering the above comments and the results, along with no obvious clinical illness, it is reasonable to assume that buffalo number 16 after being in a holding boma for just over 2 months, had no illness that should have affected its response to vaccination.

Three samples from 7 samples collected on 28 June were randomly selected for submission and significant results are tabled below, comparing results to the April results for the same animals.

Parameter	10	10	14	14	15	15
	13 and 14 /04	28/06	13 and 14 /04	28/06	13 and 14 /04	28/06
Na	149	141	143	142	151	139
K	8.30	4.9	7.50	4.7	7.90	4.7
ALT	110	93	125	105	106	117
ALP	353	104	214	157	230	141
Urea	0.80	7.4	0.70	9.2	1.00	8.3
ALB	25	25	26	28	25	24
GLOB	42	45	50	50	49	46
AST	284	261	232	284	194	244
GGT	37	18	13	11	41	33
LD	1778	1734	2032	2104	1942	2250
Ht	33	45	48	52	30	46
WCC	13.91	7.18	6.66	5.48	7.64	5.51
Thr	144	119	162	167	254	172
Neu	0.97	2.44	1.93	3.01	3.21	3.09/.28
Lym	12.24	4.12	3.93	2.25	2.83	1.93
Mono	0.14	0.14	0.13	0.16	0.99	0.17
Eos	0.56	0.07	0.67	0.05	0.69	0.06
Theil %	0.008	0.008	0.003	<0.001	0.001	0.002

Key:

10 = buffalo number 10, 14 = buffalo number 14, etc.

Blue highlighted data indicate a decrease that may have clinical significance

Yellow highlighted data indicate an increase that may have clinical significance

Considering the results table above for buffalo numbers 10, 14 and 15, the following comments were provided by Dr Fred Reyers:

As the reference ranges, used by IDEXX are “best-guess” ranges made up from uncontrolled data in the ISIS database and a guess as to how wide a variance to allow, a comparison with the “reference” range is likely to lead to errors in interpretation. As a result, the data in this

instance has been analysed on a comparison basis, between individual animals on the two dates blood samples were analysed.

These data confirm that the potassium values on the April samples were indeed invalid and the most likely reason for this is that the cells (especially red cells) were left in contact with the serum for too long and/or that transit temperatures were too high.

These data also, strongly suggest that on the April samples, these buffalo were in a very significant negative nitrogen balance OR that there was something in/about the samples that caused a serious under-read of the urea (the extremely low values on those samples had been confirmed by independent analysis – so the low values were a true reflection of what was in the serum tubes.).

The doubt expressed over other data such as ALT and LDH ("leak" from cells) has in these results been shown to be invalid with the values being very similar and yet the potassium values have normalised.

Though I doubt the validity of the GGT range used by IDEXX, buffalo 10 showed a decrease that very probably reflects a clinically significant effect – i.e. that the April sample values in excess of 35 U/L may well have reflected significant hepato-biliary pathology. This also implies that the current value for buffalo 15 is, at the very least, suggestive of ongoing/recurring pathology.

The leukocytosis in buffalo 10 has normalised and that is mainly due to the marked decrease in the lymphocytosis. Most transient lymphocytoses are reactive. This is also evident in buffalo 14 and 15 but to a much smaller degree.

Buffalo 14 and 15 had a significant neutropenia on the previous sample, which has normalised now.

The reduction in the eosinophil count (though not really clinically significantly high on the previous sample, is intriguing and could reflect a change in management or the presence of high cortisol/catecholamines (stress) and COULD then suggest that the drop in lymphocyte count, may also be partially (at least) attributed to stress.

The presence of piroplasms of *Theileria* spp. (possibly *T. buffeli*) appears, as suspected, to be a clinically insignificant background effect.

The increase in red cell count (Ht) in buffalo 10 and 15 is very probably clinically significant (increases of 36 and 53% in red cell mass are significant) but the reason for their low baseline values is not obvious from the data available. There certainly does not appear to be a role for theileriosis in this respect.

Considering the first samples were taken on the day of capture, after a stressful immobilisation from a chopper, it is likely that they were in a negative nitrogen balance and hence the low reading of the urea referred to in point 3 above. Comment five indicates there may well be some liver pathology present in these individuals although there was no clinical evidence for this. Comments 6 to 8 could probably all be explained by the stress response at capture and subsequently being kept in a boma which for a wild animal is a very stressful experience and also leads to a significant loss in body condition.

However, considering all these comments and the animals' clinical appearance, and looking at the SNT and ELISA results, there is no evidence that any of these changes affected the vaccine reaction. All three animals did not react markedly differently when compared to other animals in the group and so it can be assumed that whatever the process/es that were going on, it did not affect the response to vaccination significantly.

Five samples from 11 samples collected on 15 November were randomly selected for submission and significant results are tabled below, comparing results to the April results for the same animals.

Parameter	1	1	3	3	4	4	5	5	23	23
	15 and 16/04	15/11	15 and 16/04	15/11	15 and 16/04	15/11	15 and 16/04	15/11	15 and 16/04	15/11
Na									151	
K	9.80	4.6	11.8	4.73	12.9	4.58	14.0	4.91	8.00	4.4
ALT	82	84	107	118	92	147	120	114	125	100

Urea	0.70	7.1	1.00	8.1	1.40	8.7				
Glucose	11.1									
AST		384		375		456	301	1766		418
GGT	30	23					28	105	26	17
LDH	1653		1967		2627		2794		2819	
Ht	42	35	29	39	36	36	38	49	32	39
WCC	11.4	5.36	9.03	5	9.95	5.59	9.94	6.2	8.80	5.03
Thr	134	317	235	189	207	315	384	252	174	442
Neu	2.74	1.29	1.81	0.9	3.08	2.35	1.69	2.91	1.14	1.66
Lym	8.22	2.79	6.68	3.2	6.37	2.18	8.25	2.6	6.78	2.46
Mono	0.23	0.91	0.18	0.75	0.20	0.84	0.00	0.5	0.09	0.5
Eos	0.23	0.38	0.36	0.1	0.30	0.17	0.00	0.12	0.79	0.35
Theileria %	0.008	0	0.02	0	0.02	0.01	0.01	0	0.09	0.09

Key:

1 = buffalo number 1, 3 = buffalo number 3, etc.

Looking at the results of the five samples collected on 15 November for buffalo numbers 1,3,4,5, and 23, there were no specialist comments provided by IDEXX. If we consider the previous results and comments above, these results are again reported as compared to the initial results from the April sampling for the same animals. It is evident that the K levels have returned to within normal range and the initial results were thus flawed, as previously indicated. The ALT is still high but as previously discussed this is probably due to an inaccurate range value and the results are fairly consistent with the previous results. The urea levels for buffalo 1, 3 and 4 have also returned to normal range value and as discussed above the initial low results were probably due to the stress of capture. AST has increased significantly for all animals, especially buffalo number 5, which could be indicative of liver disease, however the other liver enzymes are in normal range. GGT has returned to normal range for buffalo 1 and 5, while it has increased significantly for buffalo 5 possibly substantiating the possibility of liver disease in this animal. For an unknown reason LDH was not run on these samples and thus no comparison can be made. The same comments made above for the 24 June samples would apply to these haematology and *Theileria* results.

Roan antelope

Nine samples collected on 9 March were submitted and significant results are tabled below.

Parameter	1	2	3	4	5	6	7	8	9
Na			152		152	156	148	150	155
K				5.9				5.90	
ALT	50	47	54			55	57	49	44
TSP		68			85	84			
GLOB					46	47			
Gluc									3.2
Cl			111		110				
GGT	67	51	67		60	57	93	67	52
LD	1772	1570	1661	2550	1418	1799	1577	2250	1560
Ht	41	36	37	34	35	36	38	44	22
WCC	5.97	4.89	6.51	5.70	7.34	6.30	7.55	7.26	5.54
Thr	185	312	301	280	192	222	72	506	251
Neu	1.85	0.59	2.41	2.05	2.35	3.91	2.94	3.48	3.49
Lym	3.76	3.52	3.65	3.19	4.48	2.14	4.08	3.56	1.66
Mono	0.12	0.00	0.13	0.23	0.07	0.06	0.08	0.00	0.22
Eos	0.24	0.78	0.33	0.23	0.44	0.19	0.45	0.22	0.17
Theileria %	0.01	0.02	0.004	0.09	0.06	0.01	0.005	0.005	0.03
Anaplasma%	0.00	0.002	0.002	0.11	0.005	0.008	0.001	0.002	0.02

Key:

1 = roan number 1, 3 = roan number 3, etc.

Red highlighted are very probably clinically significant

Lavender highlighted are possibly of clinical significance

Considering the results above the following comments were provided by Dr Fred Reyers:

It is evident from these data that the given reference range for ALT and GGT are not appropriate and need to be adjusted.

Roan 2 appeared to have significant clotting and a number of neutrophils were involved with the clots in the tails of the smear which would possibly affecting the differential count.
Roan 7 had large numbers of platelets in aggregates in the tails of the smear and was very likely to have a normal platelet count.

These findings reveal that there are no overall clinically worrying findings, pointing to a "herd problem".

The presence of piroplasms of *Theileria* spp justifies some concern because it is known that large numbers of roan have succumbed to theileriosis in SA. There are also significant parasitaemias with an *Anaplasma* spp erythrocyte parasite (resembling *A. marginale* as >80% of parasites are marginal but could be *A. bovis*). The clinical significance of anaplasmosis in non-domestic ruminants is unclear but the presence of the parasites is unmistakable.

I think it is no co-incidence that the two animals with the highest parasitaemias are also those with the lowest haematocrit values (roan 4 and roan 9).

All animals appeared clinically normal at capture and subsequent to relocation. Thus, despite roan 9 having a Ht slightly lower than the normal range used by IDEXX, this did not seem to be clinically significant. Taking this and the above comments into consideration, and looking at the SNT and ELISA results discussion below, there is no evidence that any of these changes affected the vaccine reaction.

Four samples collected on 11 April were submitted and significant results are tabled below.

Parameter	3	5	6	9
Na			150	
K	5.8			
ALT	47		57	
TSP				
GLOB				
Gluc				
Cl				
GGT	53	49		
LD	1317	1562	1824	1549
Ht	40	40	40	33
WCC	7.07	7.80	8.87	5.51
Thr	644	244	344	416
Neu	1.91	2.65	5.06	3.09
Lym	4.31	4.45	3.28	1.82
Mono	0.42	0.16	0.44	0.28
Eos	0.42	0.47	0.09	0.33
Theil %	0.06	0.03	0.03	0.18
Anaplasma%	Not seen	Not seen	Not seen	Not seen

Key:

3 = roan number 3, 5 = roan number 5, etc.

Lavender highlighted are possibly of clinical significance

Considering the results tabled above (the following comments were provided by Dr Fred Reyers:

There is reason to believe that roan 9's lower haematocrit is related to the relatively high parasitaemia and that this is linked to a lymphopenia – possibly reflecting stress. This animal also shows mild basophilic stippling and quite striking anisocytosis. However, there is no evidence of blast transformation of lymphocytes, as is seen in clinical theileriosis and ECF.

Despite this comment the animal adapted well to its new environment and showed no clinical sign of disease. There is also no evidence in the vaccination results to suggest that this individual reacted differently to vaccination than the other 8 animals.

Three samples collected on 24 October were submitted and significant results are tabled below, comparing results to the March and April results for the same animals.

Parameter	5	5	5	6	6	6	7	7	7
	9/3	11/4	24/10	9/3	11/4	24/10	9/3	11/4	24/10
Na	152			156	150		148		
K						6.6			6.22
ALT				55	57		57		
TSP	85			84					
GLOB	46			47					
Chloride			110			113			
Cl	110								
GGT	60	49		57			93		
LD	1418	1562		1799	1824		1577	1549	
Ht	35	40	35	36	40	39	38	33	43
WCC	7.34	7.80	5.58	6.30	8.87	5.15	7.55	5.51	4.45
Thr	192	244	411	222	344	593	72	416	463
Neu	2.35	2.65	3.12	3.91	5.06	3.35	2.94	3.09	2.22
Lym	4.48	4.45	2.18	2.14	3.28	1.34	4.08	1.82	1.69
Mono	0.07	0.16	0.28	0.06	0.44	0.46	0.08	0.28	0.49
Eos	0.44	0.47	0	0.19	0.09	0	0.45	0.33	0.04
Theil %	0.06	0.03	0.01	0.01	0.03	0.01	0.005	0.18	0.01
Anaplasma%	0.005	Not seen	Not seen	0.008	Not seen	Not seen	0.001	Not seen	Not seen

Key:

5 = roan number 5, 6 = roan number 6, etc.

Red highlighted are very probably clinically significant

Lavender highlighted are possibly of clinical significance

Results from the blood samples collected on 24 October only received a specialist comment by Dr Sandy May on the blood smear. These comments are incorporated into the below comments:

Na levels remained normal following high results from the initial samples, indicating that the initial high results were probably not clinically significant.

As for the initial buffalo results, and in line with the lab report that the samples were somewhat haemolysed, the potassium values are invalid and the most likely reason for this is that the cells (especially red cells) were left in contact with the serum for too long and/or that transit temperatures were too high. This also casts some doubt over other data such as ALT and LDH which may also "leak" from cells.

TSP and globulin levels have returned to normal after initial high values, indicating that the initial high results were probably not clinically significant.

GGT and LD levels have returned to normal.

All haematology parameters were in normal range and blood smear evaluation revealed no significant findings except for low level *Theileria* spp. Piroplasms, which have not increased since the first blood sample examination, despite the stress of capture and relocation.

9. APPENDIX II: Animal Ethics Committee Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Antibody response to vaccination of the African savannah buffalo *(Syncerus caffer caffer)* and roan antelope *(Hippotragus equinus)* with Clone 13 Rift Valley fever virus vaccine**
PROJECT NUMBER	**V114-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr P Brothers**

STUDENT NUMBER (where applicable)	**UP_90004613**
DISSERTATION/THESIS SUBMITTED FOR	**MMedVet**

ANIMAL SPECIES	**Roan Antelope**	**Buffalo**
NUMBER OF ANIMALS	**30**	**30**
Approval period to use animals for research/testing purposes		**November 2015-November 2016**
SUPERVISOR	**Prof. EH Venter**	

KINDLY NOTE:

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

APPROVED	Date	.30 November 2015
CHAIRMAN: UP Animal Ethics Committee	Signature	

S4285-15