Modelling the risks of foot-and-mouth disease outbreaks and assessing the effectiveness of vaccination in South Africa

by

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Declaration

I, Mohamed Mahmoud Sirdar, student number 26527406 hereby declare that this thesis entitled "*Modelling the risks of foot-and-mouth disease outbreaks and assessing the effectiveness of vaccination in South Africa*" is submitted in accordance with the requirements for the Doctor of Philosophy degree at University of Pretoria. This thesis is my own original work and has not previously been submitted to any other institution of higher learning. All sources cited or quoted in this research are indicated and acknowledged with a comprehensive list of references.

Sirdar

Mohamed M Sirdar

August 2020

Dedication

This work would not have been completed without success granted by Allah.
“Praise be to Allah, Lord of the Worlds”

A special gratitude to my loving parents, Sh. Mahmoud and Shadia who continually provided their moral, inspirational, spiritual, emotional and financial support.

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I dedicate this work to the soul of my late friend Hamid Hamedelneel Almahdi

(May your soul rest in peace)

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List of Abbreviations

°C	Degree Celsius
AHP	Analytical Hierarchy Process
BHK-21	Baby hamster kidney-21cells
BYT	Bovine thyroid cells
CFT	Complement fixation test
CI	Cumulative incidence
COV	Coefficient of variation
DAFF	Department of Agriculture, Forestry and Fisheries
DIVA	Differentiate infected from vaccinated animals
EBK	Empirical Bayesian Kriging
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FAO	Food and Agriculture Organisation of the United Nation
FMD	Foot-and-mouth disease
FMDV	Foot-and-mouth disease virus
GIS	Geographic information system
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HLB	Hydrophilic/Lipophilic Balance
IB-RS-2	Instituto Biologico Renal Suino-2 cells
IDW	Inverse distance weighting
IQR	Inter-quartile range
Km	Kilometre

KNP	Kruger National Park
KZN	Kwa-Zulu Natal
LFD	Lateral flow device
LISA	Local indicators spatial association
LITS	Livestock Identification traceability system
Log	Logarithm
mL	Milliliter
NASBA	Nucleic acid sequence-based amplification
NSP	Non-structural protein
OIE	World Organisation for Animal Health
OVR	Onderstepoort Veterinary Research
PABAK	Prevalence adjusted bias-adjusted kappa
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with tween 20
PCP	Progressive Control pathway
PCR	Polymerase chain reaction
PK	Primary pig kidney cell
PZV	Protection zone with vaccination
r_1-value	Antigenic vaccine matching serological relationship
RNA	Ribonucleic acid
RSA	Republic of South Africa
RT-qPCR	Real-time reverse transcriptase quantitative polymerase chain reaction

SADC	Southern African Development Community
SANParks	South African National Parks
SAT	Southern African Territories
SD	Standard deviation
SVD	Swine vesicular disease
TAD	Transboundary Animal Diseases
$TCID_{50}$	Median tissue culture infectious dose
UTM	Universal Transverse Mercator
VNT	Virus neutralization test
VP	Viral protein
VPN	Veterinary procedural notice for foot-and-mouth disease
VS	Vesicular stomatitis
WAHIS	World Animal Health Information Database
WGS	World Geodetic System

Summary

Project Title

Modelling the risks of foot-and-mouth disease outbreaks and assessing the effectiveness of vaccination in South Africa

Degree

Doctor of Philosophy in Veterinary Epidemiology

PhD Candidate

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Transboundary animal diseases such as foot-and-mouth disease (FMD) have negative socio-economic consequences that include impacts on food security. Vaccination reduces the number of susceptible animals and is one of the most important approaches for FMD control. In South Africa, FMD outbreaks in communal areas cause major livestock and human livelihood concerns; they raise apprehensions about the effectiveness of FMD control measures within the FMD protection areas. This study aimed to inform South Africa's FMD control policy by identifying the spatial and temporal distributions of FMD outbreaks, modelling the risks of FMDV outbreaks and assessing the effectiveness of vaccination in South Africa.

The first study used Cuzick-Edwards tests and Kulldorff scan statistics to detect spatial autocorrelation and spatial-temporal clusters of FMD outbreaks (2005 - 2016). The second study developed a new vaccine matching technique and assessed the vaccine-match of 41 FMD field viruses isolated from southern Africa (1991 - 2015). The third study developed a risk model integrating available risk factor information to identify high-risk areas for FMD outbreak occurrence and subsequent spread.

Four high-risk clusters for FMD outbreaks were identified, and the spatial distribution was consistent with contact between domestic animals and wildlife as the main contributor to FMD occurrence. Cattle numbers, cattle movement, location (province), vaccination status and vaccine matching were also important for FMD outbreak occurrences and spread. However, cattle weekly inspections were strongly related to FMD occurrence, which implies effective surveillance and inspection increased the likelihood of FMD detection. The new vaccine matching method provided a feasible and reliable approach that will contribute to the control of FMD in southern Africa.

Continued research is necessary to maximize the cost-effectiveness of FMD control in southern Africa.

Chapter i

Introduction

Foot-and-mouth-disease (FMD) is a contagious transboundary animal disease (TAD) that affects cloven-hoofed animals and reduces productivity of livestock (Grubman and Baxt, 2004). FMD is transmitted through direct and indirect contact between susceptible and infected animals and is reported to cause high morbidity. High mortality can also occur in young animals due to myocarditis (Bachrach, 1968).

The disease is caused by infection with FMD virus (FMDV), which belongs to the genus *Aphthovirus*, family *Picornaviridae* (Kitching et al., 2005)[1].

There are seven serotypes of FMDV: O, A, C, Asia 1 and Southern African Territories (SAT) 1, 2 and 3 (Larska et al., 2009). FMDV serotypes are further subdivided into topotypes based on nucleotide differences in the VP1 gene (Knowles and Samuel, 2003; Rweyemamu et al., 2008). Currently, 10 viral topotypes have been identified for SAT1, 14 for SAT2 and six for SAT3 (Vosloo et al., 2002; Ayelet et al., 2009; Ehizibolo et al., 2017).

FMD is considered to be one of the most important animal diseases globally, including within the southern African region, due to its effects on regional trade in livestock, wildlife and other agricultural products (Sinkala et al., 2014). In 2012, the World Organisation for Animal Health (OIE) and the Food and Agriculture Organisation of the United Nations

[1] https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/picornavirales/w/picornaviridae/707/genus-aphthovirus

(FAO) approved a Global FMD Control Strategy under their Global Framework for the Progressive Control of Transboundary Animal Diseases (FAO and OIE, 2012).

In South Africa, FMD is a controlled (notifiable) disease in accordance with the South African Animal Diseases Act (Act 35 of 1984) and the country is classified by the OIE as having an FMD free zone without vaccination (Bruckner et al., 2002; Vosloo et al., 2002).

FMD control areas are divided into three primary FMD control zones: infected, protection and free zones. The majority of the infected zone is the Kruger National Park (KNP), however, the adjacent wildlife conservation areas with the Ndumo Nature Reserve and the Tembe Elephant Park in KwaZulu-Natal Province are also considered infected.

FMD control measures limited the occurrence of disease to less than one outbreak per decade in South Africa up until the mid-20th century. However, from 2000, the number of FMD outbreaks in cattle within the protection zone increased by more than one outbreak a year (Baipoledi et al., 2004; Jori et al., 2009; Thomson et al., 2013). Prior to 2000, the most recent FMD outbreak in the free zone was during 1957 and the last outbreak in domestic animals within the FMD protection zone was in 1983 (Bruckner et al., 2002). All outbreaks in South Africa have been caused by SAT serotypes except a single serotype O outbreak that occurred in the free zone of KwaZulu-Natal Province during 2000 (Bruckner et al., 2002). The control of FMD in the protection zone with vaccination of South Africa is complicated by the antigenic variability of SAT FMDV and the uncertainty surrounding protection by currently used vaccines (Sirdar et al., 2019).

The livestock sector in South Africa is one of the important contributors, both socially and economically, to the lives of rural people (FAO, 2012). Livestock are raised on 80% of the

agricultural land and contribute 10% to the agricultural export industry as well as being a primary income generator for rural communities (Thomson, 2008). Communal farming areas contains approximately 41% cattle, 12% sheep, 70% goat and 27% of the pig populations in the country (Moerane, 2008). In the communal areas of South Africa, small ruminants, especially goats, are more important for income generation and food security, while cattle are more typically kept for financial stability (i.e. rarely slaughtered). One of the main challenges to livestock production in South Africa is maintaining access to high value export markets including the European Union (EU), which makes it necessary to maintain internationally recognized freedom from FMD.

Currently South Africa has been challenged by an FMD outbreak outside the protection zone and lost its FMD free status in 2019. The previous outbreak in the Free Zone in 2011 caused significant damage to South Africa's livestock and game sector through both production losses and restricted international market access (De Klerk, 2012). The FMD free status from the 2011 outbreak was not reinstated until February 2014 (OIE, 2014).

Many countries around the word aim to eradicate FMD, while this might not be possible in South Africa due to the presence of African buffalo, which are the natural reservoirs of FMDV SAT serotypes. However, it might be feasible to restrict the disease to the infected areas and apply a strict and efficient strategy of control to prevent the virus from entering the FMD free zone.

The successful control of FMD relies on the co-operation of multiple stakeholder groups. Decisions related to animal disease control are typically made at regional or national levels, but the most directly affected people are the livestock farmers and the livestock officials implementing control strategies (Roberts and Fosgate, 2018). It is anticipated

that maintaining an FMD free zone in SA will have a widely beneficial outcome. Consumers will benefit from greater stability and availability of livestock products. Livestock owners will have fewer losses and greater market opportunities and the people working in other sectors of the livestock industry will have a more reliable source of products.

Chapter ii

Literature Review

2.1. Foot-and-mouth disease (FMD)

2.1.1. Introduction

Foot-and-mouth disease (FMD) was first described in Venice in 1546 (reviewed by (Sobrino and Domingo, 2001). In August 1839, it was given its current name after an outbreak in Britain (Woods, 2004). Loeffler and Frosch in 1898 described the disease as the first animal disease caused by a filterable particle; the disease was the first animal disease to be attributed to a virus (reviewed by Brown, 2003).

FMD virus (FMDV) is classified within the genus *Aphthovirus* in the family *Picornaviridae*. Seven serotypes of FMDV exist, with very limited or no cross-protection offered among serotypes. Many different topotypes have also been described within each serotype (Schrijver and Vosloo, 2011). FMD is a World Organisation for Animal Health (OIE) listed disease (https://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2020/). The disease is present in all continents except Australia, Antarctica and North America (Grubman and Baxt, 2004; Paton et al., 2009). FMD affects domestic and wild cloven hooved animals and is characterized by high morbidity with lesion development on the mouth and feet. High mortality can occur in young animals due to myocarditis (Bachrach, 1968).

FMD can be controlled by implementing regular vaccination, which has eradicated the disease in some areas of the world (Sobrino and Domingo, 2001).

2.1.2. Geographical distribution

FMD viruses are classified into seven antigenically distinct serotypes (Knowles and Samuel, 2003). FMDV serotypes are: O, A, C, Asia 1 and Southern African Territories (SAT) 1, 2 and 3 (Larska et al., 2009). The SAT serotypes were historically restricted to sub-Saharan Africa, but in recent years SAT1 and SAT2 have been identified in North Africa, the Middle East (Di Nardo et al., 2011; Valdazo-Gonzalez et al., 2012). Serotypes O and A are broadly distributed and are reported in many African countries, the Middle East, southern Asia and the Far East. Serotype C has not occurred since 2004 and serotype Asia 1 typically occurs on the Asian continent. North and Central America, Europe, Oceania and large parts of South America are free from FMD. (Knowles and Samuel, 2003; Roeder and Knowles, 2008; Brito et al., 2017).

The FMDV genome can be divided into three main functional regions: (a) the 5′ non-coding, regulatory region; (b) the protein-coding region (subdivided in L/P1, P2 and P3); and (c) the 3′ non-coding, regulatory region. P1 encodes the four capsid proteins, P2-P3 encodes non-structural proteins involved in RNA genome replication and viral maturation (Domingo et al., 2002). The viral genome is a single stranded positive sense RNA that has a high mutation rate (Drake and Holland, 1999). The structure of FMDV is non-enveloped, approximately 25 - 30 nm in diameter and is composed of 60 copies of each of four capsid proteins named VP1, VP2, VP3 and VP4 (Domingo et al., 1985).

FMDV serotypes can be subdivided into topotypes based on nucleotide differences of the VP1 gene and multiple topotypes can be defined within each serotype. FMDV serotypes and topotypes can be used to group countries into epidemiological regions (Figure 2.1)

(Knowles and Samuel, 2003; Rweyemamu et al., 2008; Paton et al., 2009; Knowles et al., 2016).

Ten viral topotypes have been identified for serotype SAT1, 14 for SAT2, six for SAT3, 11 for serotype O, three for serotype A, three for serotype C and one for Asia 1. The Asia1 serotype is considered to have the lowest genetic variation, which suggests that it might have a more recent origin (Vosloo et al., 2002; Knowles and Samuel, 2003; Ayelet et al., 2009; Ehizibolo et al., 2017).

2.1.3. Host species

All domestic and wild cloven-hoofed animals (*Artiodactyla*) are susceptible to FMDV infection and over 70 species have been documented to become infected (Thomson et al., 2003; Schrijver and Vosloo, 2011). Cattle (*Bos taurus*), sheep (*Ovis aries*), goats (*Capra hircus*) and pigs (*Sus scrofa domesticus*) are the major domesticated species that can become infected with FMDV and serve an important role in the epidemiology of the disease (Alexandersen and Mowat, 2005). Camelids have been experimentally infected with FMDV; however, there is no evidence of transmission from camelids to other domestic livestock (Davies, 2002; Wernery and Kaaden, 2004; Wernery and Kinne, 2012). Domestic water buffalo (*Bubalus bubalis*) can become infected with FMDV and might be able to transmit the virus to other species (Weaver et al., 2013).

A wide range of wild cloven-hoofed animals including sable antelope (*Hippotragus niger*), greater kudu (*Tragelaphus strepsiceros*), impala (*Aepyceros melampus*) and deer (*Cervidae spp.*) are thought to play a role in disease maintenance (Vosloo et al., 2005).

The African buffalo (*Syncercus caffer*) is susceptible to infection, can become a persistent carrier and is the natural reservoir host for SAT serotypes (Davies, 2002).

Although FMD is known as a disease of cloven-hooved animals, it occurs naturally in other animals including the hedgehog (*Erinaceus spp.*) (Riley and Chomel, 2005). Infection has also been established experimentally in a number of other species including wildebeest (*Connochaetes taurinus*), sable antelope (*Hippotragus niger*) and bush pig (*Potamochoerus porcus*) (Macaulay, 1963). However, it is unclear whether these animals are important in the epidemiology of the disease (Snowdon, 1968).

There are reports of natural infection with FMDV in small numbers of captive animals of several non–cloven-hoofed wildlife species including Asiatic elephant (*Elephas maximus*), African savannah elephant (*Loxodonta africana*), European hedgehog (*Erinaceus europaeus*), eastern gray kangaroo (*Macropus giganteus*), Brazilian tapir (*Tapirus terrestris*), Asiatic tapir (*Tapirus indicus*) and brown bear (*Ursus arctos*) (Grosso, 1957; Neugebauer, 1976; Hedger, 1981; Bhattacharya et al., 2003; Alexandersen and Mowat, 2005; Paraguison et al., 2010; Weaver et al., 2013). Free-ranging hedgehogs have been reported to be infected with FMDV when in close proximity to an outbreak in cattle (Hedger, 1981).

A range of non–cloven-hoofed species, including rodents, rabbits, moles, armadillo, hedgehogs, squirrels, marsupials, monotremes, reptiles, primates, birds, cats, and dogs have been experimentally infected with FMDV (Hedger, 1981; Alexandersen and Mowat, 2005; Weaver et al., 2013). Asiatic black bears (*Ursus thibetanus*) have been diagnosed with FMD based on clinical signs but FMDV infection was not confirmed (Officer et al., 2014).

Although African warthogs (*Phacochoerus aethiopicus*), common warthog (*Phacochoerus africanus*) and bushpigs (*Potamochoerus porcus*) are not considered to be carriers of FMDV, antibodies have been detected up to 45 days after experimental infection with SAT1 FMDV (Pinto, 2004; Weaver et al., 2013; Rout, 2016). These species might be unrecognized reservoirs of FMDV and it has been suggested that warthogs could play a role as a secondary reservoir for FMDV or function as a bridge host between African buffalo and cattle (Miguel et al., 2017).

FMD is not a zoonotic disease despite having been recovered from people. It is extremely rare for humans to be infected with FMDV during outbreaks in animals and no clinical cases have been reported (Sellers et al., 1970). However, Prempeh et al. (2001). reviewed the disease in humans and reported that FMD was zoonotic and transmissible to humans (human to human spread has not been reported). Hence, the authors mentioned that it crosses the species barrier with difficulty and with little effect. The disease in humans is not well described, but all reported cases have been in close contact with infected animals (Prempeh et al., 2001).

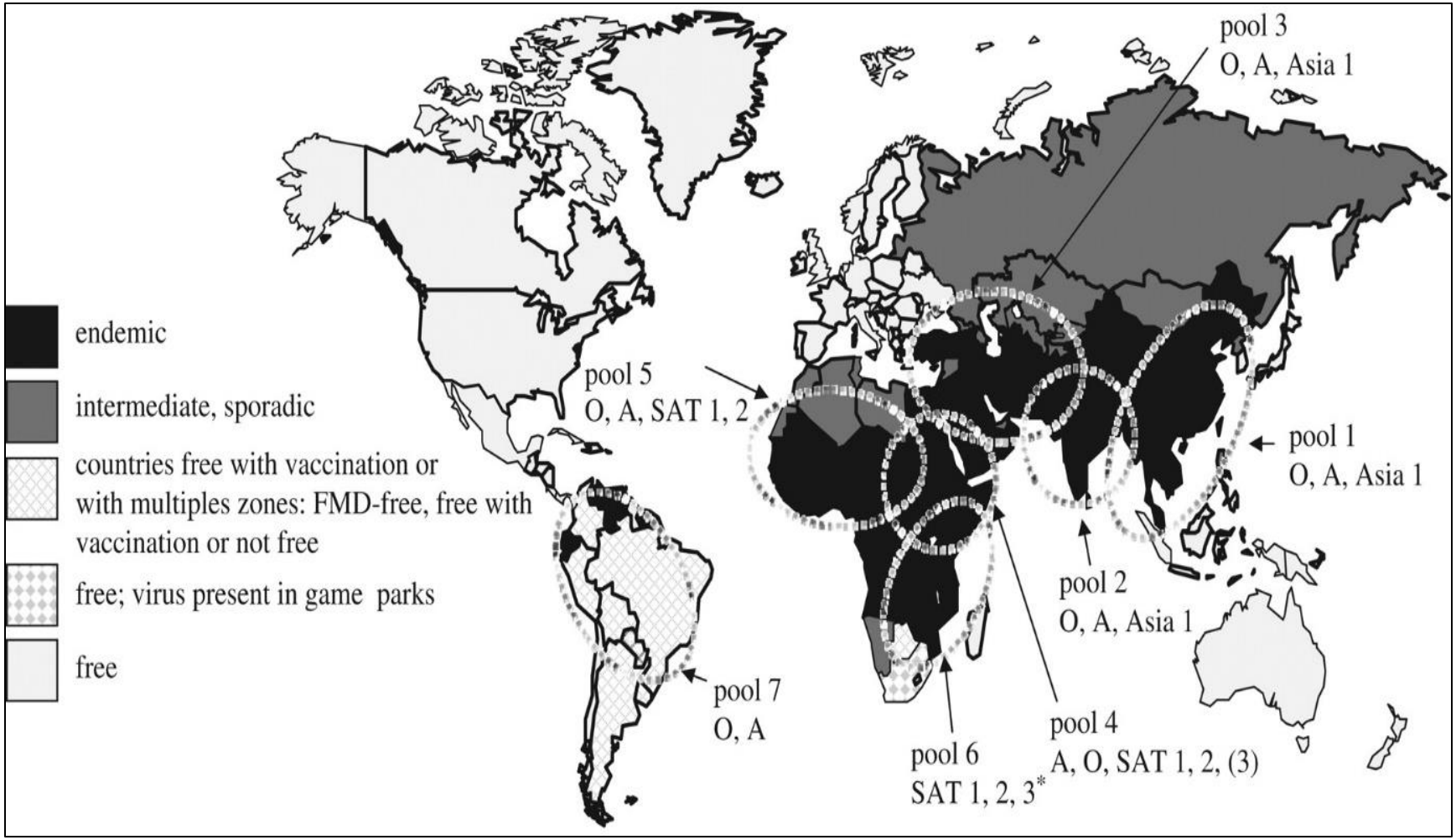

Figure 2.1: Foot and mouth disease worldwide geographical distribution[1] (Paton et al., 2009)

[1] (used with permission)

2.1.4. Transmission, carrier state and the role of small ruminants in FMD spread

Direct contact between susceptible and infected animals, contaminated fomites, or aerosols are the main routes of FMDV transmission (Alexandersen et al., 2003b). Mechanical transmission can also occur through the use of contaminated veterinary surgical instruments and artificial insemination equipment. Vehicles and humans are also considered possible sources of viral transmission (Kitching, 2002b).

Cattle, sheep and goats are highly susceptible to infection by the respiratory route but require higher viral doses for infection through the oral route (Kitching, 2002b). Calves are believed to be at risk of infection by the insufflation of milk droplets while nursing an infected dam (Donaldson, 1997; Kitching, 2002a).

FMDV can survive for weeks in moist environments at a neutral pH especially at lower temperatures. Most FMDV strains are stable within the pH range of 7.0 – 8.5. FMDV has been recovered from semen and ova of infected animals. Infected animals typically have virus in all body tissues and fluids and freezing carcases prior to rigor mortis (before pH drop) can preserve the virus (Alexandersen et al., 2002).

A carrier animal has been defined as an animal where live virus can be recovered at least 28 days post infection (Sutmoller and Casas, 2002). The virus can often be recovered from epithelial cells of the pharynx, particularly the dorsal soft palate. However, the viral titre in the oesophageal-pharyngeal fluids of carrier animals is low, and virus is not consistently recovered. In domestic cattle, the carrier state has been documented to be as long as 3.5 years, in sheep 9 months and goats 4 months. However, pigs reportedly cannot become carriers and the virus is cleared 3 - 4 weeks post infection (Alexandersen

and Donaldson, 2002). African buffalo have been reported to carry live virus for up to 5 years (Condy et al., 1985). The frequency of carrier animals in a population varies depending on species, incidence of infection, and the immune status of the herd. The proportion of carriers can vary and it is estimated to be between 15 and 50% in cattle and sheep, while in African buffaloes it can reach 70% (Condy et al., 1985). Carrier sheep and goats typically have high neutralising antibody titres (Kitching, 2002b). Cattle have been considered to be at higher risk of clinical disease compared to small ruminants presumably because of a higher respiratory volume and susceptibility to infection by the aerosol route (Alexandersen et al., 2002). However, a more recent study reported an almost similar susceptibility to FMDV infection for cattle and sheep (Bravo et al., 2015).

The role of carrier animals in the spread of virus in the field and the mechanisms for the establishment and maintenance of the carrier state are unclear. There is limited experimental evidence indicating that carrier cattle or sheep can transmit virus to uninfected animals, with an exception of a single study reporting experimental transmission from a carrier cattle to susceptible pigs (Grubman and Baxt, 2004; Tenzin et al., 2008; Paton et al., 2018). The transmission of FMDV from African buffalos to cattle has been reported in Zimbabwe and South Africa (Dawe et al., 1994; Vosloo et al., 2002). It has also been proposed that sexual transmission can occur between African buffalo and cattle, which could lead to FMDV transmission (Bastos et al., 1999).

The susceptibility of sheep and goats to FMDV infection varies by breed and viral strain (Kitching and Hughes, 2002). The respiratory tract is considered the major route of infection for small ruminants under field conditions and therefore the amount of virus excreted into the environment is important. Experimental studies have also shown that a

contaminated environment contributes considerably to FMDV transmission (Bravo de Rueda et al., 2015). Sheep and goats excrete similar amounts of airborne virus (Sellers and Parker, 1969; Donaldson et al., 1970). The maximal yields of FMDV have been obtained from sheep and goats within a few days of contact exposure (Donaldson, 1979). Sheep and goats are therefore most likely to be involved in the transmission of FMDV during the early stages of either clinical or subclinical FMDV infection. The greatest risk of transmission occurs during the initial 7 days after infection (Barnett and Cox, 1999).

2.1.5. Clinical signs

The incubation period for FMD varies by species, infecting dose, strain of virus and individual host susceptibility. Mortality in adult animals is typically low, but morbidity in naïve populations can reach 100%. In cattle, the incubation period ranges from two to 14 days with the first clinical sign being an increase in temperature (> 40°C) lasting for one to two days (Kitching et al., 2005). Disease severity can range from a subclinical infection to overt clinical disease (Schrijver and Vosloo, 2011). The clinical severity of FMD varies depending on the virus strain and the affected host species. SAT serotypes are believed to be of low virulence compared to other types. For instance, SAT viruses in African buffalo are often subclinical, while clinical expression in cattle was reported to be mild (Alexandersen and Mowat 2005; Jori et al., 2016).

The acute form of FMD is characterized by fever, depression, loss of appetite, lameness and the development of vesicular lesions. The first clinical sign is pyrexia with temperature being elevated above 40°C. Animals typically recover within 8 - 15 days after onset of clinical signs (Arzt et al., 2011a). Affected animals salivate due to vesicle development on the tongue, hard palate, dental pad, lips, gums, and muzzle. Cattle can become lame

due to vesicle development in the interdigital space and on the coronary bands (Schrijver and Vosloo, 2011). Hoof lesions usually take longer to heal and secondary bacterial infections often exacerbate clinical signs. Vesicles develop mostly in and around the mouth and on the feet but can also appear on the teats of lactating cows, as well as the prepuce, vulva and other areas. Vesicles might also be identified on ruminal pillars at post-mortem examination (Alexandersen et al., 2003b). Young animals can die due to myocarditis before developing vesicles. The syndrome is referred to as tiger heart disease due to the striped appearance of the heart muscle, and this finding might be the only clinical evidence of FMDV infection (Schrijver and Vosloo, 2011).

FMD affected cattle lose condition and losses in milk production are substantial. Reduced production often does not recover during the lactation and secondary bacterial mastitis is not uncommon (Kitching, 2002a).

The chronic form of FMD can occur four weeks after acute disease. The chronic form of the disease might be caused by mineral imbalances, which is consistent with adrenal involvement through aldosterone and several syndromes have been associated with chronic infection, including heat intolerance, pronounced panting during hot weather, increased body temperature, increased pulse rate, hirsutism and hypertrichosis that develops due to failure of seasonal shedding (Arzt et al., 2011b).

Sheep and goats are usually sub-clinically affected and clinical signs can be easily overlooked (Kitching and Hughes, 2002). However, some FMDV strains can cause severe disease in small ruminants. The incubation period of FMD in sheep and goats varies between three and eight days with viremia lasting between one and five days. FMD

clinical signs in young lambs and kids are characterised by death due to heart failure without the appearance of vesicles (Kitching and Hughes, 2002; Kitching et al., 2005).

In pigs, clinical signs include recumbency, huddling, lack of movement and lameness. The viraemic phase in pigs starts after 24 to 48 hours post contact exposure and high quantities of viral RNA are present in lingual and pedal epithelial lesions and blood (Murphy et al., 2010). Clinical signs are not obvious in adult sows and in young piglets' mortality can be the only sign of infection (Kitching and Alexandersen, 2002). Vesicles in the mouth, nose, feet and elbows might occur when animals are kept on hard surfaces (Schrijver and Vosloo, 2011).

2.1.6. Diagnosis and identification of carrier and subclinical infected animals

Clinical signs can be used to make a preliminary diagnosis of FMD. An epidemiological link to known infected premises can support this preliminary diagnosis. Confirmation depends on the detection and isolation of FMDV (Kitching, 2005). Several serological and virological methods can be used to detect antibodies to the virus, the virus itself, *i.e.* viral antigen or viral genome (Table 2.1).

2.1.6.1. Clinical diagnosis

Clinical signs become apparent between two and eight days post-infection. The clinical signs include fever, anorexia and the appearance of vesicles on the mucous membranes of the mouth including the tongue, the dental pad, gums, lips, interdigital spaces, coronary bands, muzzle, udder and teats (Arzt et al., 2011a).

Infections of the interdigital space and coronary bands in addition to stomatitis are common conditions in livestock. In some cases, it might be difficult to differentiate

between FMD and other causes based on clinical signs and gross lesions alone, necessitating further laboratory investigations (Teifke et al., 2012). Differential diagnoses include vesicular stomatitis (VS) and swine vesicular disease (SVD) (Remond et al., 2002). Some other diseases present with stomatitis including mucosal disease (bovine viral diarrhoea), malignant catarrhal fever, rinderpest (eradicated), *peste des petits* ruminants, papular stomatitis, orf, blue tongue and epizootic haemorrhagic disease (Remond et al., 2002; Teifke et al., 2012).

Pigs can be affected by FMD, VS and SVD and the vesicular lesions that develop in epithelial tissues on the feet and around the mouth might not be differentiable from each other (Fernandez et al., 2008).

The domestic hosts for VS include *Equidae* (horses, donkeys, mules) *Bovidae*, *Suidae* and South American camelids. Sheep and goats tend to be resistant with few clinical signs. In the event of horses being affected with vesicular disease then VS would be the likely diagnosis; however, laboratory diagnosis is essential when only pigs and cattle are affected because the clinical signs of VS are indistinguishable from FMD. In comparison, swine are the only host for SVD and the disease can be a subclinical, mild or severe vesicular condition. SVD must be differentiated from FMD by laboratory confirmation (OIE, 2019a).

2.1.6.2. Virological diagnosis

FMDV typing was historically done by cross-immunity testing in guinea pigs and cattle (Bachrach 1968). Limitations of the cross-immunity tests include a long time to perform, use of live animals and variable species susceptibility (Brooksby, 1949). The limitations

of cross-immunity tests encouraged the development of serological tests including complement fixation test (CFT), virus neutralization test (VNT), and enzyme-linked immunosorbent assay (ELISA). Recently, more rapid molecular techniques have been developed, which include polymerase chain reaction (PCR) (Longjam et al., 2011).

Virus isolation is typically performed using primary calf thyroid cells, which are more sensitive for virus detection than intradermal inoculation in cattle (Dekker et al., 2018). Other primary cell lines including pig, calf or lamb kidney cells can also be used for virus isolation. However, cryopreservation of primary cells after passage reduces susceptibility and these cell lines exhibit considerable inconsistency. In contrast, continuous cells (e.g., IB-RS-2 or BHK-21) when stored and maintained properly they can be used reliably for virus isolation through passaging and for other laboratory tests. Suckling mice (2 – 7 days old) are less susceptible but can also be used to isolate FMDV (OIE, 2017).

The CFT was used widely for the detection and typing of FMDV in epithelial samples from the field (Buckley et al., 1975). However, the test is relatively insensitive; it is prone to difficult interpretation due to both pro- and anti-complementary activity of samples. These problems have been eliminated by the development of antigen capture ELISA (Have et al., 1984).

2.1.6.3. Serological diagnosis

FMD can be diagnosed by the detection of a specific antibody response (Hamblin et al., 1984). Serological tests can be used for surveillance following an outbreak to identify silent infections. These tests measure antibodies to FMDV structural and non-structural proteins (OIE, 2017).

Several methods have been developed for the detection of antibodies against structural proteins including liquid-phase blocking ELISA, solid-phase competition ELISA and virus neutralisation tests (VNT). VNT is performed in microtitre plates on IB-RS-2 cells with the end-point titre calculated as the reciprocal of the last dilution of serum to neutralize 100 median tissue culture infectious dose ($TCID_{50}$) in 50% of the inoculated wells (Rweyemamu et al., 1978).

The detection of antibodies against non-structural proteins (NSP) include agar gel immunodiffusion, latex agglutination, immunoelectron-transfer blot analysis, direct ELISA and blocking ELISA. Antibody responses to the NSP 2C, 3AB and 3ABC have the potential to discriminate infected from vaccinated (using a purified vaccine in which NSP have been removed) or naive animals. The 3ABC protein is the most immunogenic of these proteins and most commonly used in assays (Remond et al., 2002).

Table 2.1: A summary of available FMD diagnostic tests

Adapted from (Compton, 1991; Longjam et al., 2011; OIE, 2017)

No	Test	Details
FMD virus/genome detection		
1	Virus isolation	A test performed using primary cell culture of bovine, ovine or porcine origin that are susceptibility to FMDV from infected tissues. Continuous cell lines from other species can also be used for virus isolation.
2	Complement fixation test	Used for typing and to distinguish different strains of FMDV
3	Antigen detection enzyme-linked immunosorbent assay	ELISAs are applied for the detection, typing and strain differentiation of FMDV isolates with better sensitivity than CFT
4	Quantitative reverse transcriptase-polymerase chain reaction	Real-time reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) tests are used as the gold standard for detecting nucleic acids from FMDV and it has become an essential tool in the research laboratory.
5	Reverse transcriptase-polymerase chain reaction	Reverse transcriptase-polymerase chain reaction (RT-PCR) is used to amplify genome fragments of FMDV in diagnostic materials including epithelium, milk, serum and oropharyngeal specimens. RT-PCR combined with RT-qPCR could have a higher sensitivity comparable to that of virus isolation.
6	Multiplex polymerase chain reaction	Designed to survey multiple regions of the genome simultaneously, thereby increasing the probability of detection.
7	Micro array-based diagnosis of FMDV	Used for the analysis of gene expression and single nucleotide polymorphisms. The method is used to type FMDV by developing an oligonucleotide microarray. It can simultaneously detect and type all seven FMDV serotypes.
8	Biosensor for detection of FMDV	It is used for FMDV diagnosis and typing. Several recombinant β-galactosidases, accommodating one or more copies of an antigenic peptide from the VP1 capsid protein of FMDV serotypes. The activity of the resulting enzymes is stimulated by antibodies directed against the viral peptide.
9	Nucleic-acid-based diagnosis method	A nucleic acid sequence-based amplification assay (NASBA) for the detection of FMDV. A continuous, isothermal and enzyme-based method to amplify single-stranded RNA.

Table 2.1: continued

No	Test	Details
Detection of FMDV immune response		
1	DIVA-based companion diagnostic approach	This approach is used to differentiate infected from vaccinated animals (DIVA) by differentiation of antibody response induced by the vaccine from those induced during infection with the wild-type virus depending on non-structural proteins (NSP).
2	Solid-phase competition ELISA	Detection of antibodies against each of the seven serotypes of FMDV
3	Liquid phase blocking ELISA	Serological test for detection of antibodies against each of the seven serotypes of FMDV
4	Virus neutralization test	A quantitative serological test for FMDV antibody that detects neutralizing antibodies.
5	Recombinant antigen-based diagnosis	A recombinant FMDV polyprotein (P1) with 3C expressed in insect cells is used to detect antibodies to FMDV using an inactivated FMDV antigen ELISA.
6	Pen-side diagnostic approach	A strip test, lateral flow device (LFD) based on a monoclonal antibody that reacts against FMDV of all seven serotypes.

2.1.7. FMD control

2.1.7.1. Control, eradication and prevention strategies

The appropriate control of FMD should be determined by considering factors related to its epidemiology (Kitching, 2005). Important factors include: i) incubation period, ii) duration of infectiousness, iii) quantity of virus particles expelled, iv) aerosolization of virus, v) survival of virus in fomites, vi) persistence of the virus in carcases, vii) existence of carriers and viii) the density and contact rate among host populations (Davies, 2002).

FMD control is aimed at preventing the spread of the virus from an infected to a susceptible animal (Orsel et al., 2005). Slaughter of infected and in-contact animals and vaccination of susceptible animals are two approaches used to control FMD (Orsel and Bouma, 2009). Slaughter can be used on its own or in combination with vaccination. Vaccination reduces the number of susceptible animals, whereas other methods used for control are to prevent the movement of the virus in infected animals, animal products, fomites or aerosols (Kitching et al., 2005). In most countries, particularly where incursions of FMD are a constant threat, regular vaccination is applied without slaughter during outbreaks. Each country's control strategy must consider OIE guidelines and all programmes should include strict controls on the movement of animals and animal products (Davies, 2002). The slaughtering of infected and contact animals should be performed in conjunction with animal movement restrictions and strict controls on the importation of animals and food products from affected areas (Saiz et al., 2002). In FMD-free countries, disease control is implemented by means of a stamping-out policy without vaccination (Knight-Jones and Rushton, 2013).

2.1.7.2. *FMD control and poverty alleviation*

Livestock are indispensable to the economies of many developing countries. Animals are sources of food, providers of income and employment and are important assets for the poorer sectors of many societies (Delgado et al., 1999). Animals are also used in rituals (thanksgiving ceremonies, marriage contracts and funerals) and for animal traction (collecting water, transport and cultivation of fields) (Personal communication). Perry and Rich (2007) evaluated the impact of FMD control on poverty reduction through the contribution of livestock to poverty alleviation. This contribution was comprised of four components: i) generating solid incentives for investment, ii) advancing international economic links, iii) offering broad access to assets and markets and iv) reducing risk and vulnerability. Quoting the same authors, these four components can be linked to FMD control by providing certain benefits to livestock producers including better access to domestic markets because the animals used for transportation will not be lame due to FMDV infection. Further benefit of FMD control are the integration of available assets for market access. Increased export revenue 'based on FMD control status' generates remarkable effects on employment and support services that raise incomes for the poor and generate additional economic growth. FMD control also strengthens the private sector through alternative markets that stimulates growth through better market access and provides support through improved livestock productivity. With that said, achieving this goal requires an enabling policy environment, diversified veterinary services and law enforcement (Perry and Rich, 2007).

2.1.7.3. FMD control in selected southern African countries

FMD control in southern Africa relies on four major measures including separation of FMD endemic areas from free zones, restricted movement of cloven-hooved animal and animal products, vaccination of cattle and surveillance. The application of these measures varies between the Southern African Development Community (SADC) countries, where the countries of Angola, Mozambique, Tanzania and Zambia have less efficient control strategies compared to Botswana, Namibia, South Africa and where systematic FMD control has been applied for decades (Thompson, 2008).

Derah and Mokopasetso (2005) reviewed the control of FMD in Botswana and Zimbabwe. However, this section will focus on Botswana due to similarities to the South African situation. Botswana implemented physical barriers (disease cordon fencing) and movement control since the early 1950s. In 1965, cattle vaccination was introduced using a bivalent vaccine containing SAT1 and SAT3 FMDV serotypes. Currently cattle in the FMD control zones are vaccinated three times a year with a trivalent vaccine. The movement of animal and animal products between disease control zones is regulated by movement protocols and official movement permits. Botswana utilises a computerised livestock traceability and identification system for cattle. Veterinary authorities emphasise disease surveillance, fencing and inspections. These measures have assisted Botswanan in gaining and retaining access to international high value beef markets (Derah and Mokopasetso, 2005, Thomson, 2008).

2.1.8. FMD vaccination

2.1.8.1. Vaccines and vaccine banks

Vaccination reduces the number of susceptible animals in a population and is regarded as one of the most important approaches for FMD control (Kitching, 2005). One of the essential components of vaccination is the degree of cross-protection provided by the vaccine against currently circulating field viruses. Thus, the FMDV used to produce the vaccine must have similar antigenic characteristics as potential outbreak strains for vaccination to be effective (Doel, 2003). Vaccination can induce protective immunity and prevent transmission in as short as four days, but the effectiveness depends upon the potency of the vaccine, the match between vaccine and outbreak strain and the level of viral exposure (Kitching, 2002b).

Diaz-San Segundo et al. (2017) reviewed vaccines currently used for FMD control. They are chemically inactivated cell-culture-derived preparations of the virus that have been blended with suitable adjuvants. Two categories of inactivated vaccines are currently available, namely: water-based and oil-based vaccines. There are two oil-based vaccines: single oil emulsions and double oil emulsions. These two types can be differentiated based on the surfactant's Hydrophilic/Lipophilic Balance (HLB) value. A surfactant having a low HLB have a high affinity for oily phases and produces water/oil (W/O) emulsions. In contrast, those with a high HLB value have a high affinity for the aqueous phase and produce O/W emulsions. On the other hand, double oil emulsions W/O/W are formed when the HLB value is intermediate (Aucouturier et al., 2001).

Aluminium hydroxide is a water-based adjuvant and FMD vaccines formulated with aluminium hydroxide are effective in ruminants but not pigs (Pay, 1984). Water-based

vaccines are usually registered for use in cattle, sheep and goats. Compared to water-based vaccines, oil-emulsion vaccinations typically induce stronger and longer lasting immunity in many species including cattle, sheep, goats, pigs and water buffalo (Barteling and Vreeswijk, 1991). FMD vaccines can include only one or multiple serotypes. Molecular techniques and recombinant technology can be used to develop alternative vaccines and these include subunit vaccines, live vector vaccines, recombinant protein and peptide vaccines, empty capsid vaccines and live attenuated vaccines (Rodriguez and Grubman, 2009).

A primary course of two inoculations two to four weeks apart is recommended for most inactivated vaccines (Ferrari et al., 2016). The frequency of revaccination depends on the epidemiological situation and the type of vaccine. Oil adjuvant vaccines are preferable when the access to animals is difficult because annual revaccination is considered sufficient to maintain protective immunity (Ferrari et al., 2016). Water based alhydrogel-saponin vaccines often require revaccination at regular intervals of four to six months to ensure protective levels of antibodies (Cox et al., 2003). FMD vaccination stimulates a predominantly humoral immune response and in order to achieve a maximum advantage from an FMD vaccine, it is necessary to ensure that the FMDV used to produce the vaccine shares antigenic characteristics with potential outbreak strains (Doel, 2003; Paton et al., 2009).

Antigen and vaccine banks are stocks of immunogenic materials that can be formulated into vaccines during emergency situations (Mumford, 2007). Vaccine banks contain concentrated antigens comprised of several subtypes and viral serotypes to ensure effectiveness against likely outbreak strains. The antigens in the banks are stored at -

130°C to guarantee potency for at least five years compared to the usual one to two years for vaccines stored at +4°C (Lombard and Fussel, 2007).

2.1.8.2. Use and application of vaccines

FMD vaccines are used for two purposes: prophylactically to protect against future infection or in an emergency situation to reduce viral transmission during an outbreak. Prophylactic vaccination on a national scale (supplemented with emergency vaccinations during outbreaks) usually targets cattle and such programmes have been successful at eradicating the disease from Europe. Eighty percent of the population should be immunized for prophylactic vaccination on a national scale, which is a broad generalisation originating from experience with other diseases in human populations (Kitching, 2002b). Cattle and pigs (in Asian countries) are routinely vaccinated for FMD in endemic countries, while sheep and goats are subjected to emergency vaccination (Cox and Barnett, 2009). Water-based vaccines are administered to cattle, sheep and goats by the subcutaneous route, usually the upper neck or in front of the shoulder. Dose volumes for large ruminants are two to three ml and small ruminants usually receive one half to one third of the cattle dose. The dose given to young animals is the same as that for adult animals. Oil-based vaccines are administered to cattle and pigs most commonly using the intramuscular route. A typical dose volume is two ml with no difference between young and adult animals (Lombard, 2012). In general, oil-based vaccines are more likely to cause vaccine reactions; reactions at the vaccination site are typically mild and hypersensitivity reactions are unlikely when the antigen and adjuvant are purified (Doel, 2003).

2.1.8.3. Vaccine matching

Vaccine matching is performed to select either the most effective vaccine for a particular circumstance or to monitor the suitability of vaccines in antigen reserves. The lack of vaccine-induced protection in the field is the practical indicator that vaccine matching is required (Alonso et al., 1993; Maradei et al., 2011).

Selection of viruses is based on epidemiological information including stages of an epidemic, location and host. In addition, viruses are selected for vaccine production based on criteria including the ability to recover high yields of stable antigen from infected cells and the immunogenicity in host species (Paton et al., 2005; Rodriguez and Grubman, 2009).

Direct vaccine matching is an *in-vivo* cross-protection test that is costly, time consuming, laborious and requires the use of live animals but is used as a direct and reliable method to measure cross-protection (Goris et al., 2007; Goris et al., 2008; Mattion et al., 2009; Nagendrakumar et al., 2011; Dekker et al., 2020). Indirect *in-vitro* methods are practical alternatives and several indirect vaccine matching tests have been developed (Paton et al., 2005). Indirect vaccine matching is typically performed by *in-vitro* serological methods and assesses the serological relationship (r_1-value) between a field isolate and a vaccine virus. Virus neutralization test (VNT) and enzyme-linked immunosorbent assay (ELISA) can be used for serological vaccine matching (Ferris and Donaldson, 1992; Kitching, 1998; Sutmoller et al., 2003).

2.1.8.3.1. Antigenic serological relationship (r_1-values)

The antigenic relationship “r” value is derived from the relationship between the reactivity of the field isolate and the vaccine strain. One-way testing (r_1) for vaccine matching is

recommended with a vaccine antiserum, rather than two-way testing (r_2) that also requires an antiserum against the field isolate to be matched (Ferris and Donaldson, 1992).

The r_1-value is calculated as the ratio of the reciprocal titre of reference serum (from vaccinated animals) against field virus to the reciprocal titre of the same reference serum against the reference vaccine virus (Paton et al., 2005; OIE, 2017).

The r_1-value is calculated using the following formula:

$$r_1 - value = \frac{\text{reciprocal titre of field isolate against vaccinated animal sera}}{\text{reciprocal titre of homologous virus against vaccinated animal sera}}$$

Although the r_1-value provides an indication of the field isolate and vaccine strain relationship, protection depends on both the cross-reactivity of antibodies elicited by the vaccine and the strength of the antibody response. The latter will be influenced by the potency of the vaccine and the number of doses given (Rweyemamu et al., 1984; OIE, 2017). Previous studies have shown that there is a strong relationship between antibody response and protection (Pay and Hingley, 1986; Hingley and Pay, 1987; Tekleghiorghis et al., 2014). A highly potent vaccine that produces a strong immune response might provide greater protection against a heterologous virus than an equally cross-reactive vaccine that stimulates a weaker immune response (Brehm et al., 2008). Booster doses of vaccine can elevate potency and the subsequent extent of antigenic coverage provided by a given vaccine, although the onset of full protection could be delayed (OIE, 2017).

2.1.8.3.2. Vaccine matching using virus neutralization test (VNT)

Virus neutralization tests (VNT) require the adaption of viral isolates for growth in cell culture. The titres of the reference serum against antigens prepared from the homologous vaccine strain and a field isolate are compared to determine how antigenically 'similar' the field virus is compared to the vaccine virus (Paton et al., 2005).

This test uses antiserum raised against a vaccine strain. The assay is performed in microtitre plates and typically employing IB-RS-2 cells, BHK-21 cells clone 13 or pig kidney cells. The end-point titre is calculated as the reciprocal of the last dilution of serum to neutralize 100 $TCID_{50}$ in 50% of the wells (Rweyemamu et al., 1978).

Stock viruses are grown and passaged in cell monolayers and stored at –70°C. The sera are inactivated at 56°C for 30 minutes before testing. The control standard serum is 21-day convalescent or post-vaccination serum. A suitable medium is Eagle's complete medium/LYH (Hank's balanced salt solution with yeast lactalbumin hydrolysate) with HEPES buffer and antibiotics. The test is an equal volume test in 50 µl amounts of the virus and the medium (OIE, 2017).

R_1-values values greater than 0.3 indicate that the field isolate is sufficiently similar to the vaccine strain and that use of a vaccine based on this strain is likely to confer protection against challenge with the field isolate (OIE, 2017).

2.1.8.3.3. Vaccine matching using ELISA

Antigens are prepared from selected strains of FMDV typically grown on monolayers of BHK-21 cells. The impurified supernatants are used and pre titrated in a two-fold dilution series without serum. The final dilution chosen is that which, after addition of an equal

volume of diluent gives an absorbance on the upper part of the linear region of the titration curve (optical density approximately 1.5). Phosphate buffer saline (PBS) containing 0.05% tween 20 and phenol red indicator is used as a diluent (PBST). A r_1-value greater than 0.4 suggests that the field isolate is sufficiently similar to the vaccine strain and is likely to confer protection against challenge with the field isolate (OIE, 2017).

2.2. FMD in South Africa

2.2.1. Introduction

FMD is a controlled disease in accordance with the South African Animal Diseases Act (Act 35 of 1984) and the country is classified by the OIE as having an FMD free zone without vaccination (Figure 2.2) (Bruckner et al., 2002). However, South Africa lost its FMD free status in January 2019 due to an outbreak outside the free zone of Limpopo Province (DAFF, 2019).

FMD control in South Africa includes animal movement restrictions placed on cloven-hoofed species and products, prophylactic vaccination of cattle, clinical surveillance, and disease control fencing to separate livestock from wildlife reservoirs (DAFF, 2014).

2.2.2. History

FMD was first officially reported in South Africa in 1892 by Hutcheon after an outbreak in Griqualand West (DAFF, 2001). Outbreaks were recognized in different parts of the country during 1893 and 1894 but no subsequent occurrences were reported between 1895 and 1903. The last FMD outbreak in the free zone prior to the 2000 outbreak (serotype O) was in 1957 (DAFF, 2001). Table 2.2 summarises the history of FMD outbreaks in South Africa.

Figure 2.2: FMD control areas of South Africa[1]

[1] (used with permission)

Table 2.2: History of foot-and-mouth disease (FMD) outbreaks in South Africa 1892-2016 (adapted from (Moerane, 2008) and OIE Disease Reports (OIE, 2014)

No	Period	Location	Serotype	Affected FMD zone	Species affected
1	1892-1894	Griqualand West	N/A[1]	N/A	Cattle, sheep, goats
2	Apr 1903	Cape	N/A	N/A	Cattle
3	Apr 1931	RSA	SAT	N/A	ND[2]
4	1957	RSA	ND	N/A	ND
5	Jul -Aug 1960	Letaba	SAT2	N/A	Cattle
6	Dec 1960	Barberton	SAT2	N/A	Cattle
7	Feb 1961	Potgletersrus & Waterberg	SAT2	N/A	Cattle
8	Jul 1961	Phalaborwa-Letaba	SAT3	N/A	Cattle
9	Oct 1967	KNP	SAT	N/A	ND
10	Jan 1968	Barberton, Letaba & Sibasa	SAT	N/A	Pigs
11	Jul 1968	Barberton	SAT1	N/A	Cattle
12	1969-1970	Barberton (Mbuzini & Masimini)	SAT	N/A	Impala, cattle[3]
13	1971-1972	KNP & Letaba	SAT	N/A	Impala, cattle
14	1973	Pilgrim's Rest & Letaba	SAT	N/A	ND
15	Aug 1974	KNP	SAT1 & 2	N/A	African buffalo
16	Feb 1975	Barberton	SAT1	N/A	Cattle
17	Jun 1977	White River & Letaba	SAT2	N/A	Cattle
18	Jul 1977-Jun 1978	Barberton, White River, Letaba, Gazankulu & Lebowa	SAT2	N/A	Cattle
19	Jul 1979	Pilgrim's Rest	SAT	N/A	Cattle
20	Nov 1979	Sibasa-Venda	SAT1	N/A	Cattle
21	Dec 1979	Glyari-Gazankulu	SAT3	N/A	Cattle

[1] N/A refers to 'not applicable'
[2] ND refers to 'no data'
[3] Cattle were affected outside KNP, while impala within the park.

Table 2.2: continued

No	Period	Location	Serotype	Affected FMD zone	Species affected
22	Jan 1980	Bolebedu-Lebowa	SAT3	N/A	Cattle
23	Apr 1981	Messina	SAT2	N/A	Cattle
24	1982-1983	KNP	SAT1 & 2	N/A	ND
25	Jul 1983	Letaba	SAT2	N/A	Cattle
26	Sept 1985	KNP & Manyeleti	SAT2	N/A	Impala
27	Nov 1985	KNP	SAT2	N/A	Impala
28	1989-1990	KNP	SAT2	N/A	Impala
29	Jul-Oct 1992	KNP	SAT2	N/A	Impala
30	Dec1995-Feb 1996	KNP	SAT2	Infected	Impala
31	Sept 2000	Camperdown-KZN[1] [2]	O	Free	Pigs
32	Nov 2000	Middleburg-Mpumalanga	SAT1	Free	Cattle
33	Dec 2000	Nkomazi-Mpumalanga	SAT1	Protection	Cattle
34	Feb 2001	Bushbuckridge-Mpumalanga	SAT2	Protection	Cattle
35	Aug 2003	Masisi-Limpopo	SAT2	Protection	Cattle
36	Jun 2004-Feb 2005	Letaba & Silwani (Limpopo)	SAT2	Protection	Cattle
37	Jul-Nov 2006	Vhembe district (Limpopo)	SAT3	Protection	Cattle
38	Sep-Oct 2009	Mbombela-Mpumalanga	SAT1	Protection	Cattle
39	Jul-Nov 2010	Ba-Phalaborwa Limpopo	SAT1	Protection	Cattle
40	Feb-Apr 2011	KZN & Gauteng[3]	SAT1	Free	Cattle
41	Jan-Apr 2012	Mbombela-Mpumalanga	SAT2	Protection	Cattle
42	Jul-Oct 2013	Limpopo	SAT1	Protection	Cattle
43	Jun-Jul 2013	Mpumalanga	SAT2	Protection	Cattle
44	Mar-Nov 2014	Mpumalanga	SAT2	Protection	Cattle
45	Dec 2015-Apr 2016	Limpopo	SAT3	Protection	Cattle

[1] South Africa lost its FMD-free status without vaccination
[2] It was the only time that Type O FMD virus was detected in South Africa
[3] South Africa lost its FMD free status without vaccination

2.2.3. The role of wildlife

Wildlife play an important role in the transmission of FMDV in South Africa. The epidemiology of the disease is complicated by the presence of the African buffalo (Tekleghiorghis et al., 2016). The African buffalo (*Syncerus caffer*) is a carrier of the SAT serotypes and the principal reservoir of infection for domestic livestock. The prevalence of FMDV infection in KNP African buffalo has been estimated to be as high as 60% and animals can be co-infected with more than one FMDV serotype (Bastos et al., 2000). However, other wildlife species, including impala, can also be a source of infection for domestic livestock (Hunter, 1998). Young African buffalo are believed to become infected at two to six months of age when maternal antibodies decline. By the time African buffalo in endemic regions reach one year of age, most animals have high antibody levels to all three SAT serotypes (Hedger, 1972; Condy et al., 1985; Sutmoller and Casas, 2002). FMDV infection in African buffalo can last for up to five years with higher viral titres in animals less than three years of age. Although transmission from African buffalo to livestock occurs through direct contact, circumstantial evidence of sexual transmission of the disease from carrier African buffalo bulls to domestic cows has been reported (Bastos et al., 1999). During this experiment, successful transmission occurred between male African buffalos and female cattle after five months of direct contact. The transmission occurred in December, which coincides with the start of the African buffalo breeding season (Dawe et al., 1994). In a follow-up experimental study, the virus was isolated from both semen and sheath washes from naturally infected African buffalo (Bastos et al., 1999; Bastos et al., 2000).

Although African buffaloes are the only known species serving as long-term maintenance hosts for SAT FMDV, several other wildlife species in southern Africa are susceptible to FMDV infection. This information can be summarised (Table 2.3) in terms of natural and experimental infection (Hedger et al., 1972; Thomson et al., 2003; Weaver et al., 2013).

2.2.4. FMD Control

South Africa implemented different FMD control policies during the period between 1931 and 2005. These policies are summarised in Table 2.4 (Moerane, 2008). Current FMD control policy includes (Thomson, 2008):

- Restriction of animal movement (African buffaloes and cattle) by fences separating FMD endemic areas from the protection zones.
- Application of a permit system to restrict the translocation of cloven-hoofed animals and animal products from endemic areas.
- Restriction of vaccinated animal movement into the free zone.
- Every four-month vaccination of cattle in the protection zone (with vaccination) against the three SAT serotypes of FMDV.
- Active and passive surveillance on an ongoing basis within the KNP to monitor disease incidence.
- Sero-surveillance to determine the immune status of animals within the vaccination areas.
- Serological testing prior to the translocation of animals and for export certification purposes.

Table 2.3: Wildlife species other than African buffalo that have been documented to be infected with FMDV within the southern African region.

No	Name	Species	Exposure and serotypes
1	Nyala antelope	*Tragelaphus angasi*	Natural
2	Giraffe	*Giraffa camelopardalis*	Natural
3	Warthogs	*Phacochoerus africanus* *Phacochoerus aethiopicus*	Natural SAT1,2 & 3
4	Impala	*Aepyceros melampus*	Natural SAT1,2 & 3 Experimental SAT1,2 & 3 & O
5	Kudu	*Tragelaphus strepsiceros*	Natural SAT1,2 & 3 Experimental SAT2
6	Wildebeest	*Connochaetes taurinus*	Natural SAT1 & 2 Experimental SAT2 & O
7	Sable antelope	*Hippotragus niger*	Natural SAT1 & 2 Experimental SAT1
8	Bush pig	*Potamochoerus porcus*	Natural Experimental SAT2
9	African elephant	*Loxodonta africana*	Natural SAT1 & A Experimental SAT1 & 2
10	Eland	*Taurotragus oryx*	Natural SAT1,2 & 3 Experimental SAT1
11	Waterbuck	*Kobus ellipsiprymnus*	Natural SAT1,2 & 3
12	Duiker	*Sylvicapra grimmia*	Natural SAT1,2 & 3
13	Grysbuck	*Raphicerus sharpei*	Natural SAT1,2 & 3
14	Reedbuck	*Redunca arundinum*	Natural SAT1,2 & 3
15	Gemsbok	*Oryx oryx gazelle*	Natural SAT1,2 & 3
16	Hartebeest	*Alcelaphus buselaphus*	Natural SAT1,2 & 3
17	Springbok	*Antidorcas marsupialis*	Natural SAT1,2 & 3
18	Bushbuck	*Tragelaphus scriptus*	Natural SAT1,2 & 3 Experimental Asia-1

Table 2.4: A summary of FMD control policy in South Africa, 1931-2005 (Moerane, 2008).

Year	Control strategy
1931	• Researching the epidemiology of the disease especially in African buffalo • Construction of game proof fences around KNP • Vaccination of cattle near the KNP and in the rest of the country (discontinued in 1957)
1956	• Implementation of the Animal Disease and Parasites Act 13 of 1956 (described control measures)
1986	• Implementation of the Animal Disease Act 35 of 1984 with its regulations as of October 1986. (Described controlled areas, stamping out strategy, vaccination of cattle and small stock, African buffalo to be kept only in controlled areas)
1987-1988	• Regulations allowing people to keep African buffaloes outside the controlled areas
1995	• Division of the country into FMD zones; FMD free zone without vaccination, infected and endemic area (KNP), buffer zone (vaccination) and surveillance zone • Application for recognition of South Africa as FMD free zone without vaccination
1997-1998	• KNP fences electrified • Reduction of the sizes of some zones
2005	• Amendment of FMD control regulations to infected zone, buffer zone (previous buffer and surveillance zones), inspection area of the free zone and free zone of the country • Stamping out with destruction, ring vaccination, physical construction of disease control fences, roadblocks, inspections and vaccination in the controlled area

In 1996, the International Committee on FMD of the OIE endorsed South Africa's FMD free status without vaccination. According to the OIE status, the areas excluded from the free zone were the endemically infected KNP and the FMD protection areas (Bruckner et al., 2002).

FMD control areas are divided into three primary FMD control zones: infected, protection and free zones (Figure 2.2). The majority of the infected zone is the KNP and adjacent wildlife conservation areas. The Ndumo Nature Reserve and the Tembe Elephant Park in KwaZulu-Natal Province are also considered infected since 2011. The KNP is a national game reserve in the north eastern part of South Africa that is approximately 480 km long and 60 to 80 km wide. The KNP and adjacent wildlife reserves are separated from communal farming areas by a 1.80 - 2.45 metre high fence (Furguson and Jori, 2010). The protection zone (approximately 480 km long and 10 - 20 km wide) is situated adjacent to the infected zone and falls within the three provinces of Mpumalanga, Limpopo, and KwaZulu-Natal. The FMD protection zone is subdivided into two areas: the protection zone with vaccination and the protection zone without vaccination. Cattle within the protection zone with vaccination are inspected for FMD at designated dip-tanks (animal assembly points) every seven days and small stock (i.e. goats, sheep and pigs) are inspected every 28 days. In this zone, cattle are routinely vaccinated every four months using a trivalent vaccine (containing SAT serotypes 1, 2 & 3) (DAFF, 2014). The protection zone without vaccination is situated to the west and south of the protection zone with vaccination and all cattle in this area are inspected every 14 days. FMD vaccination is not permitted in the protection zone without vaccination or the free zone.

The FMD regulations declared in terms of the South African Animal Diseases Act (Act 35 of 1984) provide detailed requirements for disease control, measures to be taken in the event of an outbreak and to prevent the introduction of the disease through imports of animals and animal products (Thomson, 2008). A detailed protocol (Veterinary Procedural Notice (VPN) for Foot-and-mouth Disease) was approved in 2014 describing the FMD control measures for South Africa (DAFF, 2014). The VPN's general objectives are to protect the status of the FMD free and protection zones and contain the infection within the FMD infected zones of South Africa.

According to the VPN, control measures include surveillance, vaccination and movement control and is facilitated by cattle identification. Clinical and serological surveillance are categorised based on the FMD zone. Clinical surveillance in the infected and protection zone with vaccination requires inspection of cattle every seven days and inspection of small stock (i.e. goats, sheep and pigs) every 28 days. Routine mouth examinations shall be performed and recorded on at least 10 cattle randomly selected from the presented cattle on each inspection day at every inspection point. Susceptible game species, especially impala, must be inspected as regularly as possible. Movement of livestock is only allowed from farms or dip-tanks where inspection turnout and frequency (the whole dip-tank and the herd) have been at least 50% within the last month. Serological and virological surveillance is conducted upon suspicion of FMD, or as determined by the Animal Health Section of the Department of Agriculture. Clinical surveillance within the protection zone without vaccination is performed every 14 days, while this surveillance within the high surveillance areas of the free zone is required every 28 days (DAFF, 2014).

2.3. Spatial analysis

2.3.1. Introduction

Spatial analysis is performed as an aid to the description, explanation and prediction of spatial and non-spatial data. Spatial analysis is more general than the statistical analysis of non-spatial information because it requires access to attributes, locational and topological information (Fischer and Nijkamp, 1992). Spatial analysis is often integrated with geographic information systems (GIS) and incorporated into its analytical and mapping tools. There are many aspects of basic spatial analytical techniques and procedures, which aids the analytical and modelling capabilities of GIS. Some of these techniques include: i) classification procedures for an object class of areas, ii) point pattern analysis, iii) homogeneous regionalisation procedures for an object class of areas, iv) pairing of points to generate a line object class, v) spatial correlation indices, nearest neighbour analysis, vi) spatial interaction models, shortest path procedures and optimum tour routing (Anselin and Getis, 1992; Fischer and Nijkamp, 1992).

With a disease transmission and epidemiological context, spatial and spatio-temporal proximity is a major driver for the transmission of infectious diseases, as transmission is more likely to occur if the at-risk individuals are close in a spatial and temporal sense. Spatial epidemiology mainly aims on describing spatial or geographic patterns, identifying disease clusters and explaining or predicting disease risk (Pfeiffer et al., 2008). Spatial analysis utilising FMD data could be used to study the distribution of disease and its determinants, while assisting in identifying high-risk areas for FMD occurrence, detection and spread. Spatial determinants of FMD are mainly animal densities and the distances between susceptible and infected animals (Premashthira et al., 2011).

2.3.2. Spatial interpolation

Geospatial analysis integrates spatial attributes including individual locations and contact structures among individuals to determine who is at risk of infection (Cowled and Garner, 2008). Common interpolation techniques estimate values at unsampled locations using a weighted average of nearby data (Yasrebi et al., 2009). This type of analysis can be done using GIS software to identify variables associated with epidemic progression (Rivas et al., 2003).

2.3.2.1. Inverse distance weighting (IDW)

Inverse distance weighting (IDW) and its modifications are deterministic interpolation methods. IDW estimates values at un-sampled locations based on the measurement at surrounding locations assigned weights based on distances (Yasrebi et al., 2009). Similarities among neighbours are assumed to be proportional to an inverse distance function for each location from neighbouring points. Therefore, IDW interpolation assumes that objects that are close to one another are more alike than those that are further apart. To predict a value for any unmeasured location, IDW will preferentially weight the measured values surrounding the prediction location. Thus, IDW assumes that each measured point has a local influence that diminishes with distance. The surface calculated using IDW depends on the selection of a power value (distance related function) and the neighbourhood search strategy. IDW is an exact interpolator and the maximum and minimum values in the interpolated surface can only occur at sampled points. The output surface is sensitive to clustering and the presence of outliers (ESRI, 2019b).

The IDW formula (GEODOSE, 2019):

$$X^* = \frac{W_1X_1 + W_2X_2 + W_3X_3 + \cdots + W_nX_n}{W_1 + W_2 + W_3 + \cdots + W_n}$$

x = the unknown value at a location to be determined; w = the weight (can be calculated using the next formula i.e. inverse distance of a point to each known point value that is used in the calculation); x = known point value.*

$$W_i = \frac{1}{d_{ix^*}^p}$$

P = variable stands for Power

An integrated formula can be seen below:

$$Z_p = \frac{\sum_{i=1}^{n}\left(\frac{z_i}{d_i^p}\right)}{\sum_{i-1}^{n}\left(\frac{1}{d_i^p}\right)}$$

2.3.2.2. Ordinary kriging

Kriging is an interpolation method developed from regionalized variable theory. The method defines the spatial variation of the property in terms of the variogram, and it minimizes the prediction errors, which are themselves estimated (Oliver and Webster, 1990). Kriging can also be described as a statistical method used for spatial analysis that predicts values of a continuous variable in an area (Perez et al., 2006; Yasrebi et al., 2009). It is a regression-based approach to data interpolation that uses spatial autocorrelation to produce a gradient map. Kriging procedures include simple, ordinary (stationary data), universal kriging (non-stationary data) and cokriging (group of correlated data) (Yasrebi et al., 2009).

Ordinary kriging requires the preliminary modelling step of a variance-distance relationship. The accuracy of kriging maps is dependent on the suitability of the theoretical variogram for any given data (Lu and Wong, 2008). A kriging metamodel is global and therefore covers the whole experimental area and often gives better global predictions than regression analysis. Technically, kriging gives more weight to 'neighbouring' observations (Van Beers and Kleijnen, 2003). Kriging has the advantage in that it allows the errors of the imputed values to be estimated. Kriging is based on an assumption that the spatial correlation structure is constant across locations (stationarity). However, data on disease counts, rates and risks seldom fulfils the stationarity assumption. Although it might be a major limitation for kriging, estimates are considered relatively unbiased despite non-stationarity, mis-specified variogram functions and assumptions of linear models for rate and count data (Pfeiffer et al., 2008).

Kriging as a local estimation for the unknown values of spatial and temporal variables incorporates a determination of the weight to ensure that the estimator is unbiased and that the estimation variance is minimal (Journel and Huijbregts, 1978).

Kriging formula:

$$Z_K^* = \sum_{i=1}^{n} \lambda_i z_i$$

Z^*_K = an estimate by kriging; λi = a weight for Zi; Zi = a variable.

The unbiased condition of kriging is:

$$E = \{Z_v - Z_K^*\} = 0$$

Z_V = an actual value; Z^*_K = an estimated value.

The sum of weights is:

$$\sum_{i=1}^{n} \lambda_i = 1.0$$

The kriging variance is estimated as:

$$\sigma^2 = E\{[Z_v - Z_K^*]\} = \bar{C}(V,V) + \mu - \sum_{i=1}^{n} \lambda_i \bar{C}(v_i,V)$$

$\bar{C}(V,V)$ = the covariances between sample variables; μ = Langrange parameter; $\bar{C}(v_i,V)$ = the covariances between the sample variable and the estimates

Kriging has been used by several previous FMD studies to approximate the spatial distribution of disease (Stevenson, 2003; Perez et al., 2004a, b; Lawson and Zhou, 2005; Perez et al., 2006; Highfield et al., 2008).

2.3.2.3. Empirical Bayesian kriging

Empirical Bayesian kriging (EBK) is a method that allows accurate predictions of moderately non-stationary data, which automates the most difficult aspects of building a valid kriging model. EBK automatically calculates parameters to receive accurate results through a process of sub-setting and simulations. Unlike other kriging methods, EBK accounts for the error introduced by estimating the underlying semi variogram. EBK uses

an intrinsic random function as the kriging model that requires minimal interactive modelling. “The default kriging model in EBK is termed the intrinsic random function of order 0, and the spatial correlation model is the power model where *b, c, and α* (the allowed value of the power value α is between 0 and 2) are the model parameters. This correlation model corresponds to fractional Brownian motion, also known as the random walk process. It consists of steps in a random direction and filters out a moderate trend in the data” (Krivoruchko, 2012). The standard errors of prediction are more accurate than other kriging methods, which allows for accurate predictions of moderately nonstationary data. This is especially beneficial for small datasets. However, some disadvantages of EBK include: i) processing time rapidly increases as the number of input points, the subset size, or the overlap factor increase, ii) applying a transformation will also increase processing time, iii) processing is slower than other kriging methods, especially when outputting to raster and iv) co-kriging and anisotropic corrections are unavailable (ESRI, 2019a). Although most FMD spatial studies used other forms of Kriging (Perez et al., 2006; Highfield et al., 2008), several other infectious disease studies have utilized EBK, for example: i) spatial patterns of hand-foot-and-mouth disease and its aetiology composition in China ii) predicting new locations for spatially misaligned data of a highly pathogenic avian influenza virus outbreak in Nigeria and iii) producing toxoplasmosis disease density maps in Iran (Adegboye and Kotze, 2014; Liu et al., 2015; Behine et al., 2017).

2.3.3. Spatial clustering and spatial patterns

Clustering is the spatial aggregation of disease events and is considered to represent local adverse disease risks ascribable to environmental causes (Pfeiffer et al., 2008).

Disease clustering can also be defined as a situation where there is residual spatial variation in risk after adjustment for all known predictors (Wakefield et al., 2000). The interest on clustering of diseases is due to its role in identifying common environmental factors or sources of exposure (Schwabe et al., 1977). Methods for analysing disease clustering can be divided into two main categories as specific ("local") or non-specific ("global"). The later clustering methods are used to assess if clustering is apparent throughout the study area but do not identify the specific locations of clusters (Besag and Newell, 1991).

2.3.3.1. Clustering methods

Spatial autocorrelation is present when observations from nearby locations have a more similar magnitude than expected by chance alone. The magnitude, intensity, as well as the extent of spatial autocorrelation can be quantified using spatial statistics (Fortin et al., 2013). The are several techniques available for assessing spatial autocorrelation including Moran's I, Cuzick and Edwards' k-nearest neighbouring test and Kulldorff's spatial scan statistic.

2.3.3.1.1. Moran's I (aggregated data):

Moran's I coefficient of auto correlation is similar to Pearson's correlation coefficient. It measures the similarity of an outcome variable among areas that are defined as spatially related (Moran, 1950). Moran's I is approximately normally distributed and has an expected value of -1/(N-1), when no correlation exists between neighbouring values (N equals the number of area units within a study region). A Moran's I of zero is consistent with the null hypothesis of no clustering, a positive Moran's I indicates positive spatial

autocorrelation (clustering), while a negative coefficient indicates negative spatial autocorrelation (over-dispersion).

Moran I statistic formula:

$$I = \frac{n \sum_i \sum_j W_{ij} \, (Z_i - \bar{Z}) \, \left(Z_j - \bar{Z}\right)}{(\sum_i \sum_j W_{ij} \,) \sum_k (Z_k - \bar{Z})^2}$$

Z_i = the residuals (O_i - E_i) or standardized mortality or morbidity ratio of an area (outcome of interest)

W_{ij} = measure of the closeness of areas $_i$ and $_j$

Although Moran's I was developed for continuous data, it can also be used to analyse count data. The correlation test has two disadvantages: firstly i) the assumption that the population at risk is evenly distributed across the study area and ii) the correlation or covariance is the same in all directions. Moran's I can be calculated using various software packages including R and ArcGIS (Pfeiffer et al., 2008). A model built to estimate the effect of factors associated with the spatio-temporal distribution of FMD in Tanzania used Moran's I to assess spatial autocorrelation in the model residual. The presence of spatial autocorrelation in that model led to the authors to extend the model by adding spatially structured and unstructured components (Besag and Newell, 1991; Allepuz et al., 2015). Similar approaches utilizing Moran's I static for spatial autocorrelation in FMD studies have been reported elsewhere (Bessell et al., 2010b).

2.3.3.1.2. Cuzick and Edwards' k-nearest neighbouring test (point data)

The Cuzick and Edwards' test was developed to evaluate spatial clustering when the population at risk is not homogenously distributed in space (Cuzick J., 1990). The test is

based on the locations of cases and controls from a specific region and includes a spatial scale parameter *k*. The scale refers to the number of nearest neighbours and not the geographic distance. For each case location, the test counts how many of the *k*-nearest neighbours are also cases. In situations in which disease events are spatially clustered, the nearest neighbours of cases are more likely to be cases as well.

The test statistics (T_k) is calculated as:

$$T_k = \sum_{i=1}^{n_1} m_i\ (k)$$

n_1 refers to cases and m_i (k) is the number of cases among the k nearest neighbours of case i so that $0 \leq m_i\ (k) \leq k$, for i =1,……n_1.

In the event of clustering, T_k will be large, while when all cases have controls near them T_k will be zero.

Modified (T_k) formula (population at risk data is available):

$$U_k = \sum_{j=1}^{n_1} (y_j - E_j)$$

E_j = expected number of cases; Y_j = Number of cases within each region

The advantage of this test is that it takes into account the inhomogeneous distribution of the population at risk, as cases and controls are selected from the same population (Jacquez, 1994). Limitations of the test include: i) selection of the value of the parameter *k* by the user and ii) quantitative data require categorisation as ‘case’ and ‘control’ locations causing a loss of information (Kulldorff et al., 2006b).

Although Cuzick and Edward's test was originally developed for use with point data, it can easily be adapted for aggregated data (Song and Kulldorff, 2003). Cuzick and Edwards test can be implemented using the ClusterSeer software (Pfeiffer et al., 2008). This test was used to assess the spatial autocorrelation of theileriosis outbreaks in Zimbabwe revealing that the neighbour of a theileriosis outbreak tends to be another theileriosis outbreak instead of an unaffected location (Pfeiffer et al., 1997). The same method was used to assess global spatial clustering of FMD outbreaks in Mongolia (Shiilegdamba et al., 2008).

2.3.3.1.3. Local Moran test (aggregated data)

The local Moran test identifies local spatial autocorrelation in aggregated data by disintegrating Moran's I statistics into contributions for each area within a study region (Anselin, 1995). Local indicators spatial associations (LISA), statistics for each area are calculated and these indicators detect clusters of either similar or dissimilar disease frequency values around a given observation. The sum of the LISAs for all observations is proportional to the global Moran's I statistic.

LISA statistic formula:

$$I_i = Z_i \sum_{j,j\neq 1}^{n} W_{ij} Z_j$$

Z_i & Z_j =observed values in standardised form; W_{ij} = spatial weights matrix in row-standardised form

Local Moran test to inspect the presence of spatial autocorrelation in theileriosis outbreaks by evaluating regression model residuals (Augustin et al., 1996; Pfeiffer et al., 1997).

Available software for implementing a local Moran's I tests includes ArcGIS, ClusterSeer, GeoDa, SpaceStat and R (Pfeiffer et al., 2008).

2.3.3.1.4. Kulldorff's spatial scan statistics (point data)

Kulldorff developed the spatial scan statistics (Kulldorff and Nagarwalla, 1995), which constructs a series of circles of varying radii for each sample location. Each circle absorbs the nearest neighbouring locations that fall inside it and the radius of each circle is set to increase continuously from zero until some fixed percentage of the total population is included. The programme calculates the observed number of cases (Bernoulli model) or the rate of cases (Poisson model) within each circle and then compares this value to what would be expected if the distribution were random. It is an iterative process that tests the hypothesis that for each circle there is an elevated risk of disease within the circle compared to outside (Kulldorff et al., 1998).

The test statistics formula:

$$T_{KN} = sup_z \left[\frac{O(Z)}{p(Z)}\right]^{n(z)} \left[\frac{O(Z^c)}{p(Z^c)}\right]^{n(z^c)} I\left[\frac{O(Z)}{p(Z)}\right] > \left[\frac{O(Z^c)}{p(Z^c)}\right]$$

Z^c = all circles except for Z; O = observed number of cases; p = population size in each area; I = the indicator function

The SaTScan cluster detection programme was developed by Kulldorff and integrates the spatial scan statistics into the software (Kulldorff, 1997). The software searches for clusters in datasets using two different probabilistic models: i) Bernoulli model where cases/controls status is a Boolean variable, ii) and the Poisson model where the number of cases is compared to the background population data and the expected number of cases in each unit is proportional to the size of the population at risk (Kulldorff, 1997). Circle centres are defined either by the case and control/population data or by specifying an array of grid coordinates.

Secondary clusters are computed, depending on the degree of overlap allowed in the cluster circles (Kulldorff et al., 2006a). The spatial scan statistic has been adapted to search for clusters in space and time by extending the two-dimensional circular window to that of a cylinder with time as its length.

Software for implementing and projecting the spatial scan statistic includes SaTScan, R, ArcGIS and ClusterSeer (Pfeiffer et al., 2008). Several FMD studies have utilised the SaTScan programme (Wilesmith et al., 2003; Perez et al., 2005; Shiilegdamba et al., 2008; Chhetri et al., 2010; Hayama et al., 2012; Sinkala et al., 2014; Elnekave et al., 2015; Nyaguthii et al., 2019). Spatial scan statistic was used to detect 15 significant FMD clusters by applying a Poisson model (Perez et al., 2005).

The spatial and space-time scan statistic has also been used to identify clusters with higher risk of FMD outbreaks in Nepal (Chhetri et al., 2010), Zambia (Sinkala et al., 2014), Mongolia (Shiilegdamba et al., 2008), Golan Heights of Israel (Elnekave et al., 2015) and Nakuru Country of Kenya (Nyaguthii et al., 2019).

2.3.4. Spatial risk assessment

Spatial methods can be used to assess risk to assist decision makers in developing risk management policies and strategies (Pfeiffer et al., 2008). Performing disease assessment requires georeferenced data, quantitative disease occurrence/spread information and information concerning the population at risk. These are further supplemented with data concerning risk factors and/or network data including individual animal movements (Webb, 2005). Data can be collected using different surveillance approaches including either mass, targeted, risk factor, outbreak, sentinel or syndromic surveillances (Boscoe et al., 2004).

There are several methods for disease risk assessment ranging from visualization to disease modelling. The later can be divided into data driven and knowledge driven methods. Data driven methods include statistical techniques for defining relationships between risk factors and disease, while the knowledge driven methods rely on existing knowledge about casual relationships and disease risk (Pfeiffer et al., 2008).

A common method used for risk estimation utilising quantitative data, published literature or expert opinion is a weighted linear combination (Pfeiffer et al., 2008). Risk factors can be weighted using a pairwise comparison method, where each factor is rated against all other factors and weights are calculated from these pairwise ratings (Saaty, 1980).

The Analytical Hierarchy Process (AHP) can be used to obtain ratio scales from both discrete and continuous paired comparisons using a process of relative comparisons based on human judgment (Saaty, 1987; Saito et al., 2015). These pairwise comparisons are essential in the use of AHP to establish relations within a network structure representing a problem model. AHP explicitly distinguishes and incorporates the knowledge and expertise of participants by employing their subjective judgments at every

step of process (Chelst and Canbolat, 2011). Comparisons produce dominance metrices from which ratio scales are derived in the form of principal eigenvectors or eigenfunctions, which are positive and reciprocal ($a_{ij}=1/a_{ij}$). This positive reciprocal matrix is then assessed for consistency using the consistency ratio. The next step is forming the scale of priorities (weights) by solving for the principal eigenvector of the matrix and then standardizing the results (Saaty, 1987). AHP has been used to evaluate the control of infectious bursal disease virus in California (Saito et al., 2015). Similarly, AHP has been used integrating GIS and fuzzy logic to generate hazard zones for hand-foot-and-mouth disease in Thailand (Samphutthanon et al., 2014). A modified AHP approach has also been used to estimate FMD occurrence and evaluate FMD surveillance performance in Rio Grande do Sul state, Brazil (Santos et al., 2017).

Validation of produced spatial risk maps is limited to the visual comparison with existing data or actual outbreak locations (Craig et al., 1999).

Risk factors associated with FMD introduction and spread can differ by i) disease endemicity status, ii) applied control measures, iii) type of production systems, iv) geographical location, v) social aspects, and vi) environmental factors. Several studies have estimated risk factors of farm-level transmission and FMD spread but, the literature lacks comprehensive studies on FMD risk assessment and mapping in communal farming settings (Bessell et al., 2010b).

In FMD free settings, the risk of spread has been associated with animal density, networks formed by contacts between farms, farm density, distance from the source of infection, movement of vehicles/people and road network structure (Rivas et al., 2003; Gilbert et al., 2005; Bessell et al., 2010a; Bessell et al., 2010b; Premashthira et al., 2011; Muroga

et al., 2013). FMD introduction has been mainly linked to animal movement, proximity to international borders, animal trade (legal and illegal import of livestock), airborne transmission, surveillance, wildlife and livestock density (Nissen and Krieter, 2003; Ward and Perez, 2004; Wieland et al., 2015; Santos et al., 2017). Long periods between vaccinations and infection, low effectiveness of the employed vaccine, proximity to boarders, species (cattle are highly affected by FMD outbreaks globally) and contacts with wildlife reservoirs have been reported to be significant for both FMD introduction and spread risk factors (Woolhouse et al., 1996; Elnekave et al., 2013; Elnekave et al., 2015). Within FMD endemic countries, a diverse list of risk factors for FMD introduction and spread have also been reported (Table 2.5).

Table 2.5: Reported risk factors for FMD occurrence and spread in endemic settings

FMD risk factor	Reference
Presence of a major livestock market and purchasing livestock at markets	*(Perez et al., 2004a; Megersa et al., 2009; Jemberu et al., 2016; Nyaguthii et al., 2019)*
Density of cattle herds and small ruminants	*(Perez et al., 2004b; Jemberu et al., 2016)*
Close proximity to slaughterhouses	*(Lindholm et al., 2007)*
Roads	*(Allepuz et al., 2015)*
Proximity to borders and cross border movements	*(Megersa et al., 2009; Allepuz et al., 2015)*
Use of a shared bull	*(Nyaguthii et al., 2019)*
The number of animals sourced from other farms	*(Nyaguthii et al., 2019)*
Use of communal dipping	*(Nyaguthii et al., 2019)*
Number of other species sharing the same farm	*(Nyaguthii et al., 2019)*
Production system	*(Jemberu et al., 2016)*
Adjacency to a national park	*(Jemberu et al., 2016)*
High herd mobility, and movement of infected animals	*(Megersa et al., 2009)*
Draught	*(Megersa et al., 2009)*
Intermingling of cattle at grazing areas and water sources	*(Cleland et al., 1996; Megersa et al., 2009; Dukpa et al., 2011; Nyaguthii et al., 2019)*
Landscape characteristics and heterogeneity of the Environmental landscape	*(Dion, 2011, 2012),*
Fence damage, maintenance, and permeability	*(Dion, 2012; Mogotsi et al., 2016)*
Contacts between African buffalos/livestock	*(Jori and Etter, 2016; Miguel et al., 2017)*
Herd immunity	*(Jori et al., 2009; Jori and Etter, 2016)*
Human population	*(Dion, 2011)*
Elephant density	*(van Schalkwyk et al., 2016)*
African buffalo density	*(van Schalkwyk et al., 2016)*
Flooding events	*(van Schalkwyk et al., 2016)*
River crossings	*(van Schalkwyk et al., 2016)*

Chapter iii

Spatial distribution of foot-and-mouth disease (FMD) outbreaks in South Africa (2005-2016)

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3.1. Abstract

Foot-and-mouth disease (FMD) is a transboundary animal disease that has negative socio-economic consequences including impacts on food security. In South Africa, FMD outbreaks in communal farming communities cause major livestock and human livelihood concerns; they raise apprehensions about the effectiveness of FMD control measures within the FMD protection areas. This study aimed to identify high-risk areas for FMD outbreaks at the human/domestic animal/wildlife interface of South Africa. Cuzick-Edwards tests and Kulldorff scan statistics were used to detect spatial autocorrelation and spatial-temporal clusters of FMD outbreaks for the years 2005 - 2016.

Four high-risk clusters were identified and the spatial distribution was consistent with human activities in addition to wildlife contacts as main contributors of FMD occurrence. Strategic allocation of resources, focused control measures and cooperation between the affected provinces are recommended to reduce future outbreaks. Further research is necessary to design cost-effective control strategies for FMD.

Keywords: Cluster; Dip-tank; Human/domestic animals/wildlife interface; Kruger National Park; Risk

3.2. Introduction

Foot-and-mouth-disease (FMD) is a contagious trans-boundary animal disease that affects cloven-hoofed animals and reduces productivity of livestock (Grubman and Baxt, 2004). The disease is caused by FMD virus (FMDV), which belongs to the genus *Aphthovirus* within the family *Picornaviridae* (Kitching et al., 2005). There are seven serotypes of FMDV: O, A, C, Asia 1 and Southern African Territories (SAT) 1, 2 and 3 (Larska et al., 2009).

Wildlife plays an important role in the transmission of FMDV in southern Africa due to African buffalo (*Syncerus caffer*) being a carrier of SAT serotypes and the principal reservoir of infection for domestic livestock (Vosloo et al., 2002; Brahmbhatt et al., 2012). However, other wildlife species, including impala (*Aepyceros melampus melampus*), can be a source of infection for domestic livestock (Hunter, 1998).

In South Africa, FMD is a controlled disease in accordance with the South African Animal Diseases Act (Act 35 of 1984) and the country is classified by the World Organisation for Animal Health (OIE) as having an FMD free zone without vaccination (Bruckner et al., 2002). However, South Africa lost its FMD free status in January 2019 due to an outbreak outside the protection zone of Limpopo Province (DAFF, 2019). FMD control in South Africa includes animal movement restrictions placed on cloven-hoofed species and products, prophylactic vaccination of cattle, clinical surveillance and disease control fencing to separate livestock from wildlife reservoirs (DAFF, 2014).

FMD control areas are divided into three primary FMD control zones: infected, protection and free zones. The majority of the infected zone is the Kruger National Park (KNP) and adjacent wildlife conservation areas with the Ndumo Nature Reserve and the Tembe Elephant Park in KwaZulu-Natal Province considered infected. The KNP and adjacent

wildlife reserves are separated from communal farming areas by a 1.80 - 2.45-metre high fence (Furguson and Jori, 2010). The protection zone (approximately 480 km long and 10 - 20 km wide) is situated adjacent to the infected zone and falls within the three provinces of Mpumalanga, Limpopo, and KwaZulu Natal (DAFF, 2014). The FMD protection zone is subdivided into two areas: the protection zone with vaccination and the protection zone without vaccination. Cattle within the protection zone with vaccination are inspected for FMD at designated dip-tanks (animal assembly points) every seven days and small stock (i.e. goats, sheep and pigs) are inspected every 28 days. In this zone, cattle are routinely vaccinated (every four months) using a trivalent vaccine (containing SAT serotypes 1, 2 and 3) (DAFF, 2014). The protection zone without vaccination is situated to the west and south of the protection zone with vaccination and all cattle in this area are inspected every 14 days. FMD vaccination is not permitted in the protection zone without vaccination or the free zone.

FMD control measures limited the occurrence of disease to less than one outbreak per decade in South Africa up until the mid 20th century. However, from 2000, the number of FMD outbreaks in cattle within the protection zone increased by more than one outbreak a year (Baipoledi et al., 2004; Jori et al., 2009; Thomson et al., 2013). Prior to 2000, the most recent FMD outbreak in the free zone was during 1957 and the last outbreak in domestic animals within the FMD protection zone was in 1983 (Bruckner et al., 2002). All outbreaks in South Africa have been caused by SAT serotypes except a single serotype O outbreak that occurred in the free zone of KwaZulu-Natal Province during 2000 (Bruckner et al., 2002).

FMD outbreaks in southern Africa and other endemic areas support the collection of qualitative and quantitative data in an effort to strengthen FMD control measures. The objective of this study was to identify high-risk areas for FMD outbreaks in the protection zone of South Africa that could be used to inform FMD control policy. A secondary objective was to determine if the distance between dip-tanks and wildlife areas was associated with the detection of FMD.

3.3. Material and methods

3.3.1. Study area

The study was performed in the FMD protection zone with vaccination (PZV) in the South African provinces of Mpumalanga and Limpopo (Figure 3.1). The FMD PZV of Mpumalanga and Limpopo Provinces includes four and six local municipalities, respectively. These study areas are regarded as the KNP human/wildlife/livestock interface adjacent to the FMD infected zone. The study excluded the PZV of KwaZulu-Natal Province since this is a relatively recently designated protection area (2014) and FMD outbreaks have not been recorded since its establishment.

3.3.2. Data collection and management

All reported FMD cases in domestic cattle from 1 January 2005 to 31 December 2016 in the PZV communal farming areas for both Limpopo and Mpumalanga Provinces of South Africa were identified (Appendix A-1 & A-2). This time period was chosen due to the availability of data from the World Animal Health Information Database (WAHIS). The unit of analysis (case) was defined as any dip-tank where at least a one domestic bovine showed FMD clinical signs. All clinical outbreaks required laboratory confirmation of at

least one animal using liquid-phase blocking ELISA and confirmed by either PCR or virus isolation.

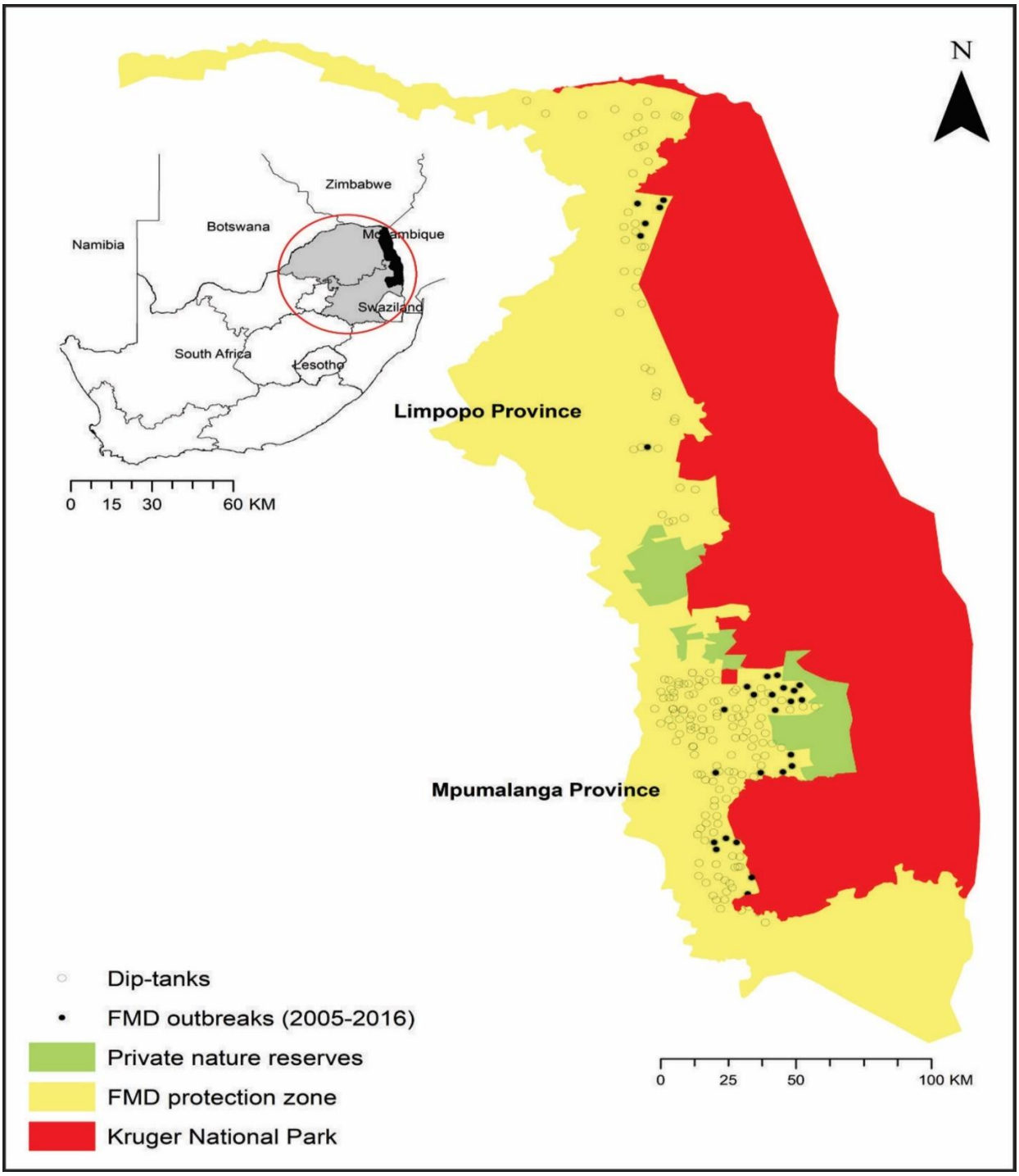

Figure 3.1: South Africa's Limpopo and Mpumalanga Provinces FMD control zones (infected and protection), animal dip-tanks and FMD outbreaks 2006-2016 (no outbreaks occurred during 2005)

Dip-tanks are animal assembly points used for routine inspection and disease control and these were the statistical unit of analysis. A dip-tank serves at least one village within an average area of five km^2. Commercial farms (large scale and industry driven organised farming) within the PZV were excluded from analysis. The Department of Agriculture and Rural Development, Veterinary Services of both Mpumalanga and Limpopo Provinces provided information on all registered dip-tanks including georeferenced locations, total susceptible animals and animal-specific demographics. Animal demographics were extracted from monthly FMD inspection reports. These reports include information on the total number of cattle per dip-tank at the beginning of each month as well as increases (births and in-movement) and decreases (death, out-movement and slaughter) of the population (Appendix A-3 & A-4).

FMD cases in domestic cattle were aggregated per dip-tank and summed for the total time length of each outbreak. The length of an outbreak was defined as the elapsed days between first and last reported cases based on the OIE database (http://www.oie.int/animal-health-in-the-world/the-world-animal-health-information-system/data-after-2004-wahis-interface/).

Coordinates for dip-tanks were converted to the Universal Transverse Mercator (UTM) zone 36S World Geodetic System (WGS) 1984 format and plotted using ArcGIS version 10.4 (ESRI, Redlands, California, USA).

3.3.3. Descriptive analyses

The cumulative incidence (CI) of affected cattle at the dip-tank level was used as the dependent variable for some statistical analyses. The cumulative incidence was calculated as the total number of reported FMD cases occurring within each dip-tank for

a specific outbreak divided by the total number of susceptible cattle reported in the WAHIS reports. Data were assessed for normality by plotting histograms, calculating descriptive statistics, and performing the Anderson-Darling test for normality. Data violating the normality assumption were $\log_{10}$ transformed prior to statistical analysis.

The distance from each dip-tank to the nearest fence of a wildlife reserve was estimated using the measuring tool in the GIS software. All dip-tanks were divided into two groups; either affected at some time or never experiencing a FMD outbreak during the study period. These distances were compared between outbreak and non-outbreak dip-tank groups using a Mann-Whitney U test. Statistical analysis was performed using SPSS 24.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and results were interpreted at $p < 0.05$.

3.3.4. Spatial interpolation

The average number of cattle for January and December 2009 (year corresponding to the mid-point of the study period) was used to describe the spatial distribution of the susceptible cattle population. The average cattle population was then modeled using a point density approach, which calculates a magnitude-per-unit area from point features that fall within a neighborhood around each cell (http://pro.arcgis.com/en/pro-app/tool-reference/spatial-analyst/point-density.htm). The FMD CI was interpolated using ordinary kriging (Waller L.A., 2004; Stevens et al., 2009). The CI were analysed per serotype and a combined analysis of all SAT serotypes was also performed. All maps were produced in ArcGIS 10.4.

3.3.6. Spatial cluster analyses

Cuzick and Edwards tests (Cuzick J., 1990; Selvin et al., 2004) were used to estimate global spatial autocorrelation. The Euclidean distances between all dip-tanks were

calculated using the easting-northing UTM coordinates (eq. 1). The nearest neighbouring dip-tank was identified, and each neighbouring pair was classified based on the presence/absence of reported FMD outbreaks during the study period. For example, a case/case pair was defined as a dip-tank and its nearest neighbor when both dip-tanks experienced FMD outbreaks at any time during the study period. The observed frequency of case/case pairs of nearest neighbors (*m*) was then compared to the expected frequency (E [*m*]) (eq. 2). In Eq.2, *p* represents the probability of the occurrence of a case/case pair, n_1 represent the total number of cases and n represents the total number of dip-tanks (controls + cases = $n_0 + n_1 = n$). The test statistic (eq. 3) was calculated for the null hypothesis assuming a hypergeometric distribution.

$$\sqrt{(E1 - E2)^2 + (N1 - N2)^2} \quad \text{.....(eq.1)}$$

Expected frequency = $E\,[m] = np = n\,\frac{(2^{n1})}{(2^n)} = \frac{n1\,(n1-1)}{n(n-1)}$ ..... (eq. 2)

$$z = \frac{m+0.5-E\,[m]}{\sqrt{np\,(1-p)}} \quad \text{.....(eq. 3)}$$

Clustering of FMD outbreaks in the PZV was evaluated using purely spatial, purely temporal and space-time scan statistics. Tests were performed using SaTScan v9.4 software [http://www.satscan.org/] based on a Bernoulli probability model (dip-tank affected yes/no) following the method described by Kulldorff and Nagarwalla (Kulldorff and Nagarwalla, 1995; Estrada et al., 2008). The spatial range of the space-time scan analysis included all dip-tanks in the FMD PZV and the time range was the 12 years from 2005 to 2016. The null hypotheses were that the distribution of affected dip-tanks and the

time frame of infection were random. Statistical significance for the identification of clusters was set as $p < 0.05$.

3.4. Results

There were a total of 201 dip-tanks within the PZV during the study period. In Mpumalanga Province, 91 329 cattle were distributed between 151 dip-tanks and in Limpopo Province, 42 417 cattle were distributed between 50 dip-tanks at the mid-point of the study period (2009). The highest cattle densities in the study area were observed in the northern part of Mpumalanga Province (Figure 3.2).

A total of 1040 cattle FMD cases were reported during the study period. These cases occurred within seven outbreaks and all outbreaks were due to infection with SAT serotypes (Table 3.1). In total, thirty-one dip-tanks were affected. Two dip-tanks within Limpopo Province experienced two independent FMD outbreaks each during the study. Four outbreaks and almost 75% (23/31) of the affected dip-tanks were in Mpumalanga Province.

SAT2 FMD outbreaks were more common in Mpumalanga and all SAT3 FMD outbreaks occurred in Limpopo Province. Descriptively, outbreaks in Mpumalanga Province took longer to resolve (range 7-18 months and 4-6 months for Mpumalanga and Limpopo respectively; Table 3.1) and a higher proportion of affected cattle were reported.

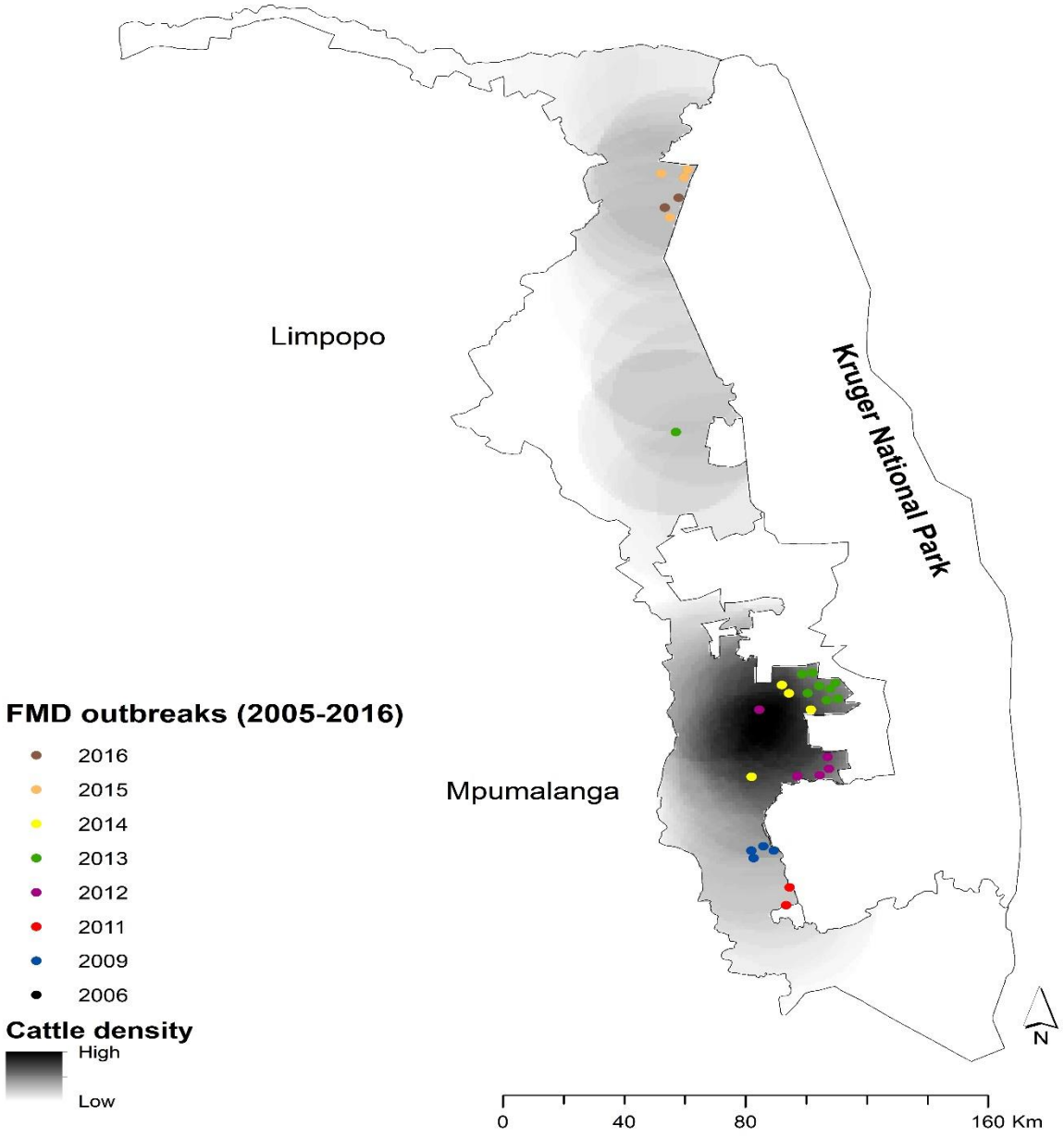

Figure 3.2: Point density estimation of 2009 cattle population in the protection zone with vaccination of South Africa in relation to 2006-2016 FMD outbreak locations (no outbreaks occurred during 2005).

Table 3.1: FMD outbreaks within the South Africa FMD protection zone with vaccination, duration and seasonal comparisons (2005-2016)

Outbreak ID	Province	Duration (months)	Start of outbreak	End of outbreak	Dip-tanks affected (n)	Serotype (SAT[1])	Total susceptible animals	Total cases	Proportion of affected animals
1	Limpopo	5	07/2006	11/2006	2	3	1300	42	0.03
2	Mpumalanga	9	09/2009	05/2010	4	1	9505	757	0.08
3	Mpumalanga	8	12/2011	07/2012	2	2	5510	38	0.007
4	Mpumalanga	4	04/2012	07/2012	5	2	1750	16	0.009
5	Limpopo	6	07/2013	12/2013	1	1[2]	1141	1	0.0008
6	Mpumalanga	19	08/2013	02/2015	12	2	42903	131	0.003
7	Limpopo	7	12/2015	06/2016	5	3	6060	55	0.009
					31		68169	1040	0.015

[1]Southern African Territories.

[2]This outbreak affected other dip-tanks located in the FMD protection zone without vaccination (not part of the study area).

The distance from a dip-tank to a wildlife reserve fence was shorter for the dip-tanks that experienced FMD outbreaks. The median (range) distances for FMD outbreak and non-outbreak dip-tanks were 2.5 km (0.1-10.9) and 8.4 km (0.1-57.9) respectively ($p < 0.001$). The kriged CI were higher in Mpumalanga Province and the northern area of Limpopo Province (Figure 3.3).

The spatial distribution of FMD affected dip-tanks appeared different based on FMDV serotype. SAT1 and SAT2 outbreaks tended to occur in southern and northern Mpumalanga respectively, while SAT3 was in the northern part of Limpopo Province (Figure 3.4).

There was significant global spatial autocorrelation ($p <0.001$) and the study identified two spatial and four temporospatial clusters of FMD outbreaks (Figure 3.5 a, b). This spatial autocorrelation was attributed to the local transmission after disease introduction. Three of these clusters were detected in Mpumalanga Province with the other being in the northern part of Limpopo. Three of the four high rate clusters were close to a major road, while rivers crossed two high rate areas (Figure 3.6). Most of the outbreaks occurred during the period 2012-2015 and the temporal model identified a single high rate cluster for the years 2012-2015 (Table 3.2).

Table 3.2: Spatio-temporal clusters of FMD outbreaks in South Africa (2005-2016)

Year	Location	Cluster dip-tanks (n)	Radius (Km)	Observed cases	Cases in area (%)	Expected cases	Relative risk	P-value
Purely spatial								
High rate								
2005-2016	Mpumalanga	16	15.1	11	68.8	2.4	6.4	0.001
2005-2016	Limpopo	4	8.4	6	100	0.9	7.9	0.002
Low rate								
2005-2016	Mpumalanga	91	30.6	2	2.2	13.9	0.1	0.002
2005-2016	Mpumalanga	47	20.8	0	0.0	7.2	0.1	0.010
Space-time								
High rate								
2009	Mpumalanga	5	5.8	4	80.0	0.1	65.4	0.002
2012	Mpumalanga	4	7.9	4	100	0.1	81.7	0.002
2013	Limpopo	11	8.1	8	72.7	0.2	69.6	0.001
2015	Mpumalanga	4	8.4	4	100	0.1	81.7	0.002
Low rate								
2006-2010	Mpumalanga	99	44.1	0	0	7.1	0	0.029
Purely temporal								
High rates								
2012-2015	-	201	NA	22	2.7	11.3	4,28	0.002
Low rates								
2007-2008	-	201	NA	0	0	5.6	0	0.016

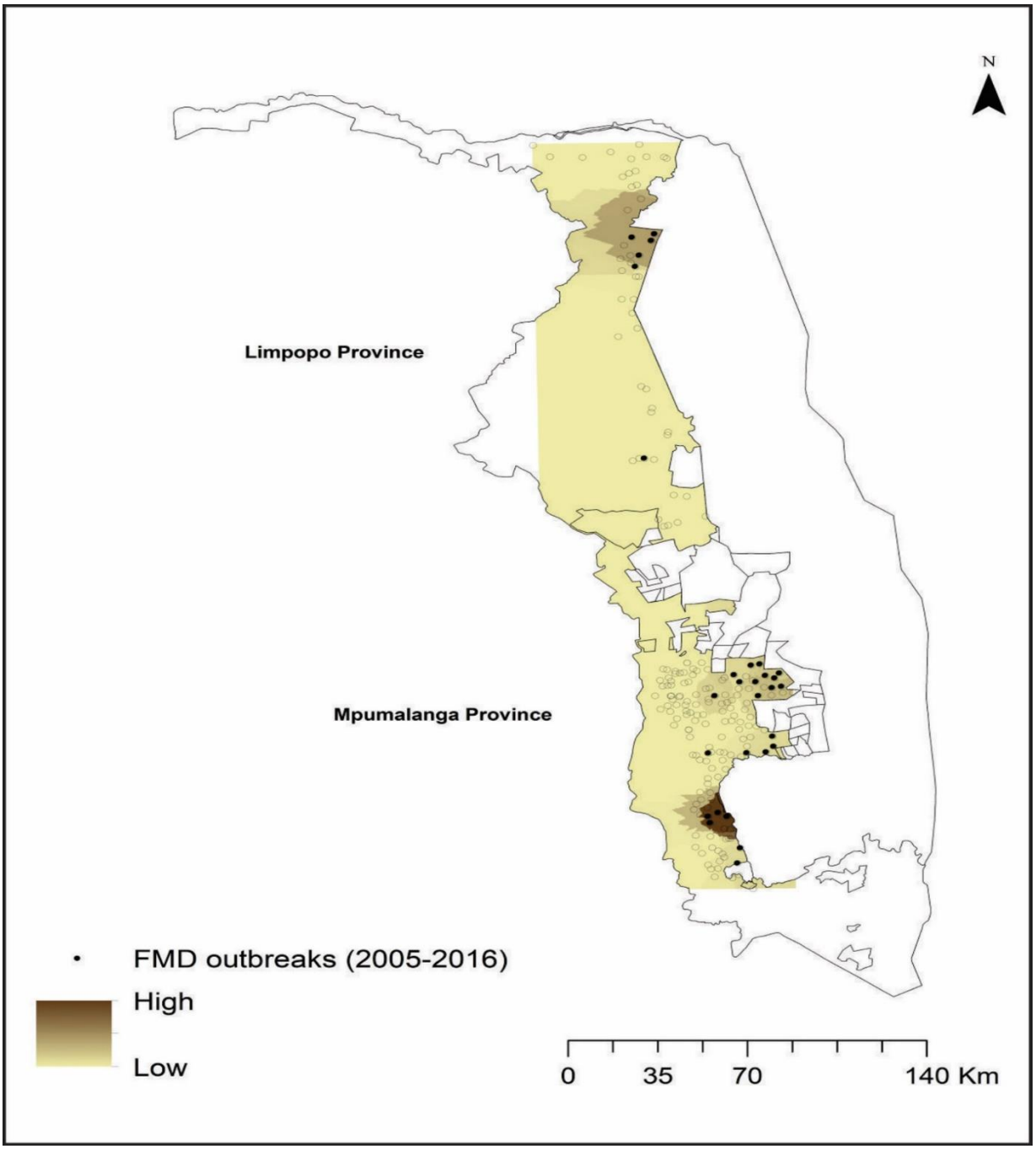

Figure 3.3: Kriged FMD cumulative incidences of 2006-2016 cattle outbreaks in the protection zone with vaccination of South Africa (no outbreaks occurred during 2005).

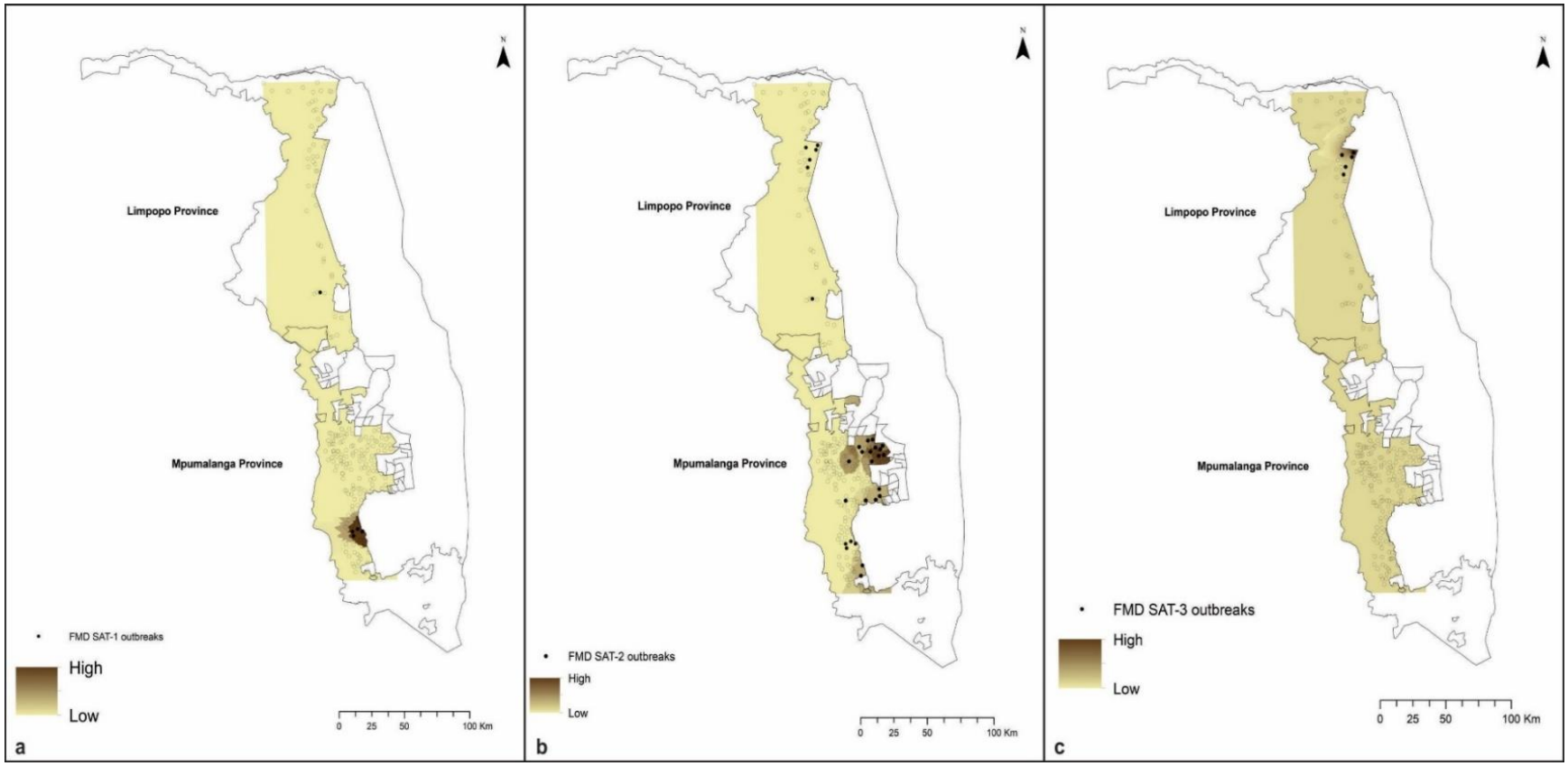

Figure 3.4: Kriged FMD cumulative incidences of 2005-2016 SAT1 (a), SAT2 (b), and SAT3 (c) cattle outbreaks in the protection zone with vaccination of South Africa.

Figure 3.5: a) Space-time, and b) spatial high rate clusters of FMD outbreaks in cattle (2005-2016) in the protection zone with vaccination of South Africa.

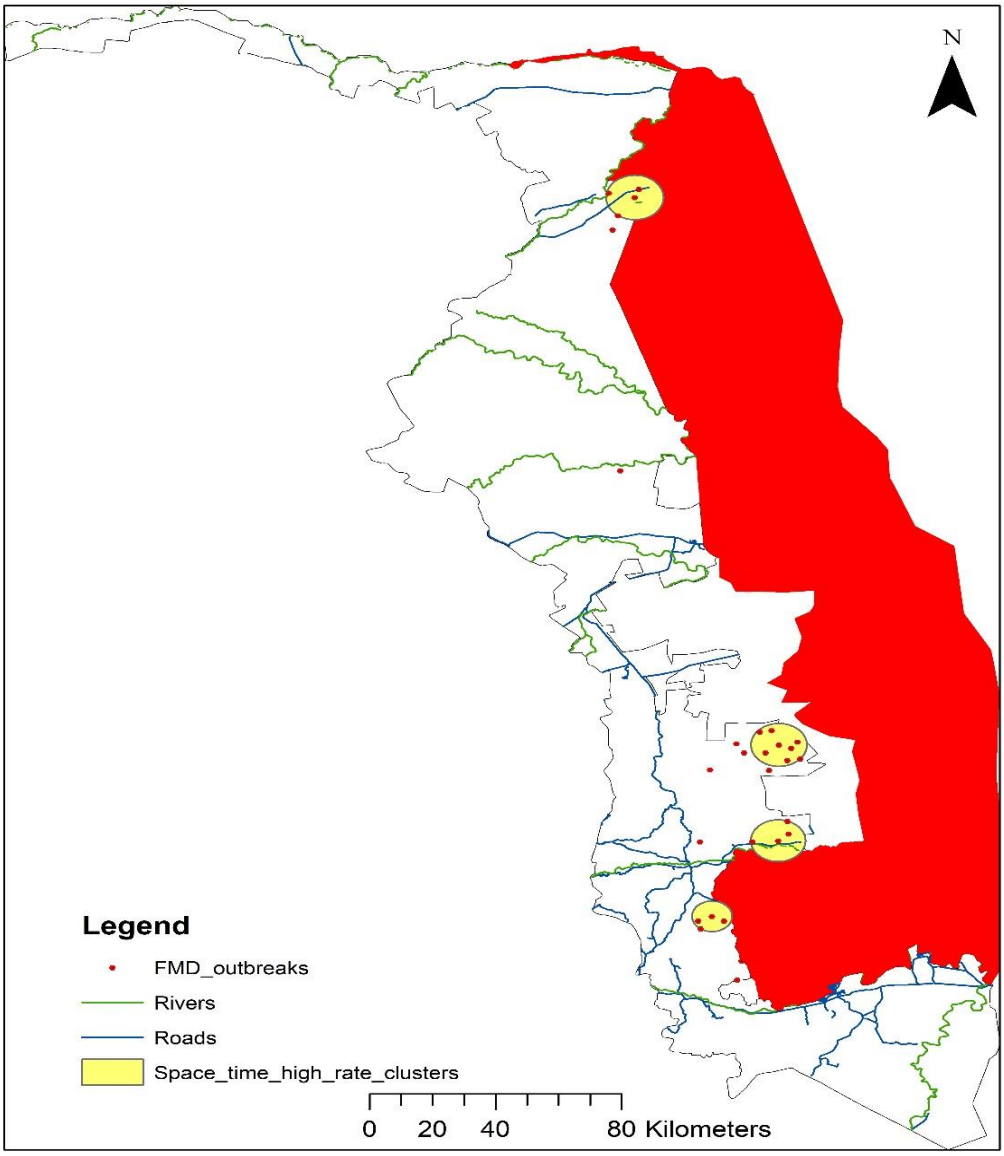

Figure 3.6: Space-time high rate clusters of FMD outbreaks (2006-2016) in cattle including roads and rivers in the protection zone with vaccination of South Africa.

3.5. Discussion

Dyason (2010) previously summarized FMD outbreaks that had occurred in South Africa between 1970 and 2009. In the 34 years prior to our study period (1970-2004), seven SAT1, 14 SAT2 and one SAT3 FMD outbreaks were detected in cattle. No FMD outbreaks were identified in the protection zone between 1983 and 2000. However, six outbreaks occurred in cattle between 2000 and 2008. Five of these outbreaks were epidemiologically linked to contact with African buffalo due to close proximity between wildlife and livestock in the PZV (van Schalkwyk et al., 2016). During our 12-year study period, three SAT2 outbreaks and two outbreaks for SAT1 and SAT3 serotypes occurred in the study area (Limpopo and Mpumalanga Provinces). The proportion of affected cattle was lower in Limpopo Province compared to Mpumalanga and this might be due to a lower cattle density in affected dip-tanks of Limpopo. Sixteen percent (8/50) of Limpopo dip-tanks were affected compared to 21% (31/151) in Mpumalanga during the study period arguing against a difference in risk between provinces ($\chi^2 = 0.49$; $p = 0.48$). The typical duration of a South African outbreak was descriptively longer than reports in Northern Hemisphere countries. The median duration of FMD epidemics in cattle herds was 67 days in FMD free countries that applied depopulation with or without vaccination (Halasa et al., 2015). This should be compared to seven months for South Africa. The longer duration of FMD outbreaks might be attributable to the unrestricted movement of livestock within the disease management area (village/dip-tank; 3-6 km^2), which typically constitutes 50-200 livestock owners. In contrast to control measures in Europe, stamping-out is not practiced during an outbreak.

The total number of outbreaks in our study was too small to formally test for temporal and seasonal effects. However, 57% (4/7) of outbreaks were reported during the dry season

(April - September). This finding is consistent with other studies (Jori and Etter, 2016; van Schalkwyk et al., 2016) that reported a high concentration of stray African buffalos around the KNP fence at this time. This finding is also consistent with Dyason's (Dyason, 2010) summary where the majority (70%) of outbreaks reported during 1970 - 2009 were between June and October. Brahmbhatt et al (2012) also reported that contact between cattle and African buffalo was higher during April - September. During the dry season, villagers might graze their livestock in KNP increasing the chance of wildlife and domestic animal contact (Jori et al., 2011; Brahmbhatt et al., 2012). Our finding was consistent with these previous studies, but the small sample size prevented statistical confirmation.

Significant global spatial autocorrelation of affected dip-tanks in the PZV during 2005 - 2016 was evidence of a non-random distribution of FMD outbreaks. The cumulative incidence, duration of each outbreak and the total number of affected dip-tanks was higher in Mpumalanga compared to Limpopo ($\chi^2 = 7.26$; $P < 0.001$). Mpumalanga had a high density of dip-tanks in close proximity to each other with high numbers of cattle in the province. The highest number of cattle were in the northern and southern area of Mpumalanga and the far north east of Limpopo. Descriptive results suggest a link between cattle densities and FMD outbreaks with higher cattle densities increasing the chance of an FMD outbreak (Figure 3.2). This finding is consistent with previous research suggesting that cattle population density is positively associated with the risk of FMD outbreaks (Allepuz et al., 2015).

In the study area, the majority of private game reserves are either inside or adjacent to the PZV, increasing the chance of wildlife/domestic animal contact. In all the outbreaks that were recorded in this study, the first dip-tank to be infected was the closest dip-tank

to the disease control fence; subsequently affected dip-tanks were always further away. This is circumstantial evidence that outbreaks were a consequence of wildlife/cattle contacts possibly due to fence permeability. Proximity to national parks and potential wildlife reservoirs were previously reported to increase the risk of FMD occurrence in other African countries (Allepuz et al., 2015). The western fence of the KNP has different structural types, and thus susceptible to different degrees and causes of damage (Bengis R.G., 2003). A section of KNP fence was damaged by a flood in 2000 and has been without functional electrification (Furguson and Jori, 2010) for much of its length. This fence remains highly permeable to cattle and African buffalo in certain areas (Jori et al., 2011). The most influential factor for spatial and temporal risk of FMD transmission is the effectiveness of wildlife and cattle separation (Dion, 2012).

SAT1 and SAT2 kriged outbreak surfaces suggested higher risk in Mpumalanga Province, while SAT3 was predicted to occur only in Limpopo Province (Figure 3.4). Historically, SAT3 was detected in cattle of this province in 1979 and again in 2002 within a African buffalo herd outside KNP (Dyason, 2010). SAT3 has not been reported in Mpumalanga suggesting that it is circulating within a defined population in Limpopo Province. SAT3 FMD outbreaks have been detected in African buffalo of South Africa but not impala. In contrast, more than 28 SAT1 and SAT2 FMD outbreaks have been reported in impala since 1970 (Dyason, 2010).

The areas of high rate clustering (Figure 3.5) occurred at the same locations as high cattle populations. In addition, dip-tanks in these hot spot areas were close to each other and in close proximity to game reserve fences. The only cluster that was identified in Limpopo included the two dip-tanks that experienced FMD outbreaks twice during the study period.

Three of the hot spot areas contained a road network that could influence the control of animal movements (Figure 3.6), while two high-risk areas included rivers (Figure 3.6).

In Tanzania, the risk of FMD is associated with proximity to main roads (Allepuz et al., 2015). Also, the movement of livestock along major roads contribute to the persistence of FMD during epidemic phases in Iran (Perez et al., 2005). Disease control fences that are near or crossed by rivers can be damaged during floods or not provide a secure barrier during times of extreme drought. The spatiotemporal dynamics of cattle/African buffalo contacts are influenced by animal interaction, landscape and KNP fence breakage (Dion, 2011). Severe drought and animal congregation increase the risk of FMD outbreaks and spread within similar endemic settings (Shiilegdamba et al., 2008). Identified hot spot areas require intensive monitoring and maintenance of the disease control fence to reduce contact between wildlife and livestock.

A limitation of this study was the use of maps based on spatial interpolation to identify high- risk areas. Spatial distances were calculated using straight line or Euclidean distances, which failed to capture the biological realism of disease spread. Spatial interpolation and cluster analyses are also data driven approaches and prone to sampling bias. Such analyses might miss areas of high-risk because of gaps in surveillance efforts (Escobar and Craft, 2016). All animals within a dip-tank are seldom examined during an outbreak. Despite this fact, the identified cases were used to calculate cumulative incidences using the total number of animals in the population rather than the total number of animals examined (data are not recorded or reported). Another potential bias is that surveillance efforts might not have been uniform and FMD detection might have been biased towards dip-tanks with more cattle.

3.6. Conclusion

The identification of high rate clusters can be used to support the implementation of risk-based surveillance and thus mitigate the FMD outbreak risk at wildlife interfaces of southern Africa. Wildlife-livestock contact, cattle density and road networks appear to play a role in FMD occurrence suggesting that animal movement and human activities might be drivers of FMD transmission that require further study. This study provides preliminary findings and the development of quantitative models could further assist with targeted FMD surveillance and control. Improved control is expected to lead to a more robust rural economy that would contribute to poverty alleviation in the region.

Chapter iv

A novel method for performing antigenic vaccine matching for foot-and-mouth disease in absence of the homologous virus[1]

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https://www.sciencedirect.com/science/article/pii/S0264410X19308801?via%3Dihub

4.1. Abstract

Foot-and-mouth-disease (FMD) is a contagious transboundary animal disease that has negative consequences on regional and international trade. Vaccination is an important approach for FMD control and an essential consideration is the degree of cross-protection conferred by the vaccine against currently circulating field viruses. The objective of this study was to evaluate a new vaccine matching technique that does not require knowledge concerning the homologous vaccine virus. As a proof of concept, the vaccine-match was assessed for 41 FMD field viruses isolated from southern Africa over a 25-year period.

A diverse group of 20 SAT1 and 21 SAT2 FMDV isolates collected from cattle and wildlife during 1991-2015 were selected for this study. Virus neutralization tests were performed against two sets of pooled sera for each serotype: vaccinated cattle sera (4 to 16 weeks post-vaccination) and convalescent cattle sera (3 weeks post-experimental infection). Novel r_1-values were calculated as the ratio of the titre of the vaccinated sera to the titre for convalescent cattle sera. A validation r_1-value was calculated based on an assumption concerning the true homologous vaccine virus. There was a strong positive correlation between r_1-values for the novel and the validation methods for SAT1 viruses (Spearman's rho = 0.84, $P< 0.01$) and a very strong correlation for SAT2 viruses (Spearman's rho = 0.90, $P< 0.01$). In addition, there was moderate to good agreement between the novel and validation methods for both serotypes based on a r_1-value cut-off of 0.3, which is presumed to represent a good vaccine-match. The agreement between methods using prevalence-adjusted and bias-adjusted kappa (PABAK) was 0.67 and 0.84 for SAT1 and SAT2 viruses, respectively.

The new r_1-value method provides a feasible, alternative vaccine matching approach that could benefit FMD control in southern Africa.

Keywords

FMD, Southern African Territories (SAT), vaccine matching, r_1-value, VNT

4.2. Introduction

Foot-and-mouth-disease (FMD) is a contagious transboundary animal disease (TAD) that affects cloven-hoofed animals and reduces productivity of livestock (Grubman and Baxt, 2004). The Southern African Development Community (SADC) has categorized FMD as one of the most important animal diseases within the region due to its effects on regional trade in livestock, wildlife and other agricultural products (Sinkala et al., 2014).

FMD causes high morbidity with vesicular lesions developing on the mouth and feet. High mortality can occur in young animals due to myocarditis (Bachrach, 1968). The disease is caused by FMD virus (FMDV), which belongs to the genus *Aphthovirus*, family *Picornaviridae* (Kitching et al., 2005). The viral genome is a single-stranded positive sense RNA that has a high mutation rate. The structure of FMDV is non-enveloped, 27 nm in diameter and is composed of 60 copies of four capsid proteins named VP1, VP2, VP3 and VP4 (Domingo et al., 1985).

There are seven serotypes of FMDV: O, A, C, Asia 1 and Southern African Territories (SAT) 1, 2 and 3 (Larska et al., 2009). The SAT serotypes were historically restricted to sub-Saharan Africa, but in recent years SAT1 and SAT2 viruses have been identified in North Africa and the Middle East (Di Nardo et al., 2011; Valdazo-Gonzalez et al., 2012). For the FMDV serotypes, major virus lineages have evolved separately and cluster according to their geographic location that can be subdivided into topotypes based on nucleotide differences of VP1 sequences (Knowles and Samuel, 2003; Rweyemamu et al., 2008). Ten viral topotypes have been identified for SAT1, 14 for SAT2 and six for SAT3 (Vosloo et al., 2002; Rweyemamu et al., 2008; Ayelet et al., 2009; Valdazo-Gonzalez et al., 2012; Ehizibolo et al., 2017).

Vaccination reduces the number of susceptible animals and the level of viral shedding in a population and is regarded as one of the most important approaches for FMD control (Kitching, 2005; Bravo et al., 2015). The essential component of vaccination is the degree of cross-protection provided by the vaccine against currently circulating field viruses. Thus, the FMDV used to produce the vaccine must have similar antigenic characteristics as potential outbreak strains for vaccination to be effective (Doel, 2003). Vaccination can induce protective immunity and prevent infection by exposure to FMDV aerosols in as short as four days but the effectiveness depends upon the potency of the vaccine, the match between vaccine and outbreak strain and the level of viral exposure (Kitching, 2002b).

Vaccine matching is performed to select either the most effective vaccine for a particular circumstance or to monitor the suitability of vaccines in antigen reserves. The lack of vaccine-induced protection in the field is the practical indicator that vaccine matching is required (Alonso et al., 1993; Maradei et al., 2011). Direct vaccine matching is an *in vivo* cross-protection test, which is costly, time consuming, laborious and requires the use of live animals (Goris et al., 2007; Goris et al., 2008; Mattion et al., 2009). Indirect *in vitro* methods are practical alternatives and several indirect vaccine matching tests have been developed (Paton et al., 2005). Indirect vaccine matching is typically performed by *in vitro* serological methods and assesses the serological relationship (r_1-value) between a field isolate and a vaccine virus. Enzyme-linked immunosorbent assay (ELISA) and virus neutralization test (VNT) can be used for serological vaccine matching (Ferris and Donaldson, 1992; Kitching, 1998; Sutmoller et al., 2003). The r_1-value is calculated as the ratio of the reciprocal titre of reference serum (from animals exposed to the vaccine virus)

against field virus to the reciprocal titre of the same reference serum against the reference vaccine virus (Paton et al., 2005; OIE, 2017).

In South Africa, FMD is a controlled animal disease in accordance with the South African Animal Diseases Act (Act 35 of 1984) and the country had been classified by the OIE as having an FMD free zone without vaccination (Bruckner et al., 2002). However, the FMD free status has been suspended due to a recent SAT2 outbreak (OIE, 2019b). FMD control in South Africa includes animal movement restrictions placed on cloven-hoofed species and products, prophylactic vaccination of cattle, clinical surveillance, and disease control fencing to prevent contacts between livestock and wildlife (DAFF, 2014).

The control of FMD in the protection zone with vaccination of South Africa is complicated by the antigenic variability of SAT FMDV and the uncertainty surrounding protection by currently used vaccines. The objective of this study was to develop and evaluate a new vaccine matching technique that does not require the live homologous vaccine virus in the laboratory to perform the vaccine matching. A secondary objective was to estimate the serological vaccine match for 41 FMD field viruses isolated from southern Africa during 1991-2015.

4.3. Material and Methods

4.3.1. Study area

FMD control areas in South Africa are divided into three primary FMD zones: infected, protection and free. The majority of the infected zone is comprised of the Kruger National Park (KNP) and adjacent wildlife conservation areas. The protection zone is adjacent to the infected zone and falls within the three provinces of Mpumalanga, Limpopo, and Kwazulu-Natal. The FMD protection zone is subdivided into two areas: the protection zone with and without vaccination. Cattle within the protection zone with vaccination are prophylactically vaccinated every four months using a trivalent vaccine (containing SAT serotypes 1, 2 & 3).

The study area included the FMD infected and protection zones in the provinces of Mpumalanga and Limpopo (Figure 4.1). The study excluded the protection zone of Kwazulu-Natal Province because it is a recently designated protection area (2014) and no FMDV isolates had been obtained prior to the study. Ethical approvals were obtained from the Animal Ethics Committees of the University of Pretoria (No. v005-15) and Onderstepoort Veterinary Research of the South African Agricultural Research Council (No.25/04/P001) (Appendix B-1).

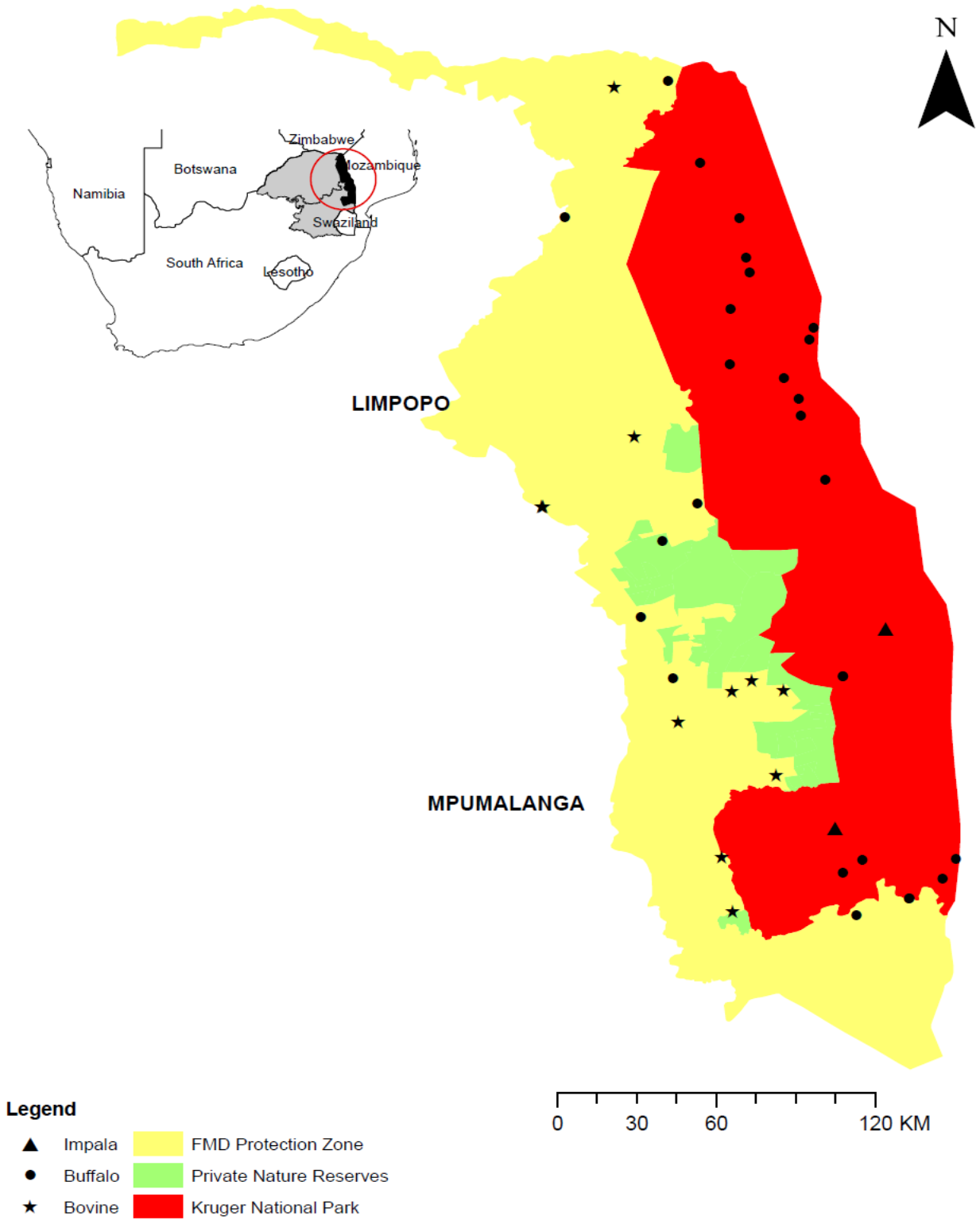

Figure 4.1. Location and animal species of FMD study viruses isolated in South Africa between 1991-2015

4.3.2. Cattle immunization and sera collection

Vaccinated animal sera were collected in a previous study (Lazarus et al., 2018). Cattle were vaccinated against FMDV by the South African veterinary services using a trivalent inactivated-vaccine containing SAT1, SAT2 and SAT3 antigens (Aftovax®, Merial Animal Health Limited /Botswana Vaccine Institute, Gaborone). Cattle were longitudinally sampled and tested for antibodies against FMDV structural proteins using the liquid-phase blocking ELISA (Hamblin et al., 1986). One hundred and ninety-one sera samples from 136 cattle at least 4 weeks post-vaccination and with ELISA titres ≥ 2.2 $\log_{10}$ were selected to provide sufficient quantity of sera for the current study. Separate serum pools were created for SAT1 (92 samples of 0.5 ml each) and SAT2 (99 samples of 0.5 ml each) serotypes. Serum pools were negative for non-structural proteins (NSP) using the commercial PrioCHECK® FMDV NS Antibody ELISA Kit. Testing was performed following manufacturer instructions with slight modifications (Brocchi et al., 2006).

4.3.3. Cattle viral infection and sera collection

Eight cattle were housed at the animal containment BSL-3 facility at Transboundary Animal Diseases-Onderstepoort Veterinary Research (TAD-OVR). Convalescent sera were obtained from cattle 21 days post-infection with SAT1 (SAR/10/10; SAR/21/10; SAR/08/10) and SAT2 (SAR/4/14; SAR/1/13; SAR/15/13) field viruses by two consecutive FMDV challenge studies. Eight cattle in total were infected; two per serotype for the first and second passage, respectively. For the first passage, cattle were inoculated intradermolingually with three SAT1 viruses isolated during a single outbreak in cattle (2010; FMD protection zone with vaccination). The three viruses were combined into a single pool prior to infection. The same infection procedures were performed with three

SAT2 viruses also from a single outbreak in cattle (2013-2014; FMD protection zone with vaccination). FMDV from infected tissue collected from the first study was isolated and pooled and inoculated into a second set of cattle for a second passage. The experimental infection dose was 10^4 to 10^6 median tissue culture infective dose ($TCID_{50}$) for both passages and serotypes.

Blood was collected at day 21 post-challenge (study termination) and serum samples stored at -70° C until used. Convalescent sera from both passages were subsequently pooled independently by serotype and stored at -20°C.

4.3.4. FMD virus selection

Eight (4 SAT1 and 4 SAT2) reference FMDV from the virus bank of the TAD-OVR FMD Reference Laboratory were included in the study. Six of the 8 available reference strains were selected for sera standardisation. Reference viruses were collected from South Africa and other southern African countries. Reference strains were possible vaccine strains, with four of the viruses historically used to control FMD along the borders of South Africa (Maree et al., 2010). The FMDV used in this study were either isolated in bovine thyroid cells (BTY) or primary pig kidney cells (PK). They were further propagated and passaged in baby hamster kidney-21 cells clone 13 (BHK-21) and Instituto Biologico Renal Suino-2 cells (IB-RS-2) respectively.

A diverse group of 20 SAT1 and 21 SAT2 field viruses were chosen for antigenic vaccine matching. The FMDV were purposely selected to represent all cattle reported outbreaks during the study period and to include genetically diverse viruses based on available VP1 sequence data. Selected viruses were isolated from the KNP and the South African FMD

protection zones. Viruses were propagated in IB-RS-2 cells to a titre of > 4.5 log_{10} /ml (3 – 8 passages) and stored at -70°C until used.

4.3.5. Serological testing

Virus neutralization tests (VNT) were performed as described in the 2018 OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (adopted 2017) (OIE, 2017). The assay was performed in microtitre plates on IB-RS-2 cells with the end-point titre calculated as the reciprocal of the last dilution of serum to neutralize 100 $TCID_{50}$ in 50% of the wells (Rweyemamu et al., 1978). Neutralization titre determinations were performed in duplicate and the test was repeated when the coefficient of variation between duplicates was ≥ 30%. VNT were performed for field and reference viruses against both the challenged/convalescent and vaccinated cattle sera pools (Appendix B-4).

4.3.6. Sera pool standardisation

Prior to performing antigenic vaccine matching, VNT were performed for three SAT1 and three SAT2 reference viruses to standardize the FMDV antibody concentrations between the challenged/convalescent and vaccinated cattle sera pools (Appendix B-2 & B-3). It was expected that convalescent sera would have higher antibody concentrations and therefore the initial plan was to dilute the convalescent pool to match the antibody concentration of the vaccinated cattle pool. However, the standardisation procedure suggested that both pools had similar antibody concentrations and consequently the vaccine matching analysis was performed without diluting the sera.

4.3.7. Antigenic vaccine matching

One-way antigenic relationships (r_1-value) were calculated (Rweyemamu, 1984) with r_1-values greater than 0.3 indicating that the vaccine is likely to confer protection against challenge with the field isolate (OIE, 2017). Two approaches to vaccine matching were employed.

- An "anticipated r_1-value" was calculated following the usual method by making an assumption concerning the true vaccine (homologous) virus. The available reference viruses were screened against vaccinated animal sera and the virus with the highest recorded titre was chosen as the presumed homologous vaccine virus.
- A "novel r_1-value" was developed as a newly proposed method to calculate r_1-values by comparing the titre from pooled vaccinated sera to a standardized positive control.

The "anticipated" r_1-value was calculated based on an assumed homologous vaccine virus using the following formula:

$$\frac{\text{titre of vaccinated animal sera against the field isolate}}{titre\ of\ vaccinated\ animal\ sera\ against\ the\ assumed\ homologous\ virus}$$

"Novel" r_1-values were calculated by substituting the usual r_1-value denominator with a standardized positive control. This approach is similar to the use of a sample to positive ratio, which has been described previously (Pare et al., 1995; Lanyon et al., 2013). The novel r_1-value was calculated as the ratio between the heterologous virus against the vaccinated animal sera and the homologous infection virus against post-infection sera:

$$\frac{\text{titre of vaccinated animal sera against the field isolate}}{titre\ of\ post-infection\ sera\ against\ homologous\ infection\ virus}$$

4.3.8. Statistical analysis

The genetic diversity of selected SAT1 and SAT2 FMD viruses was evaluated using phylogenetic analysis. Partial VP1 sequences published for the viruses were assembled and aligned using BioEdit 5.0.9 and MEGA version 5 software packages, respectively (Hall, 1999). A neighbor-joining tree was constructed in MEGA 5 using the p-distance method with bootstrap values of the phylogenetic nodes being calculated out of 1000 replicates (Tamura et al., 2011).

The correlation between novel and anticipated r_1-values were assessed using scatter plots and Spearman's rank correlation coefficients. Correlations were categorized as ≤ 0.35 low or weak correlation, 0.36 - 0.67 modest or moderate correlation, 0.68 - 0.89 strong correlation and ≥ 0.9 very strong correlation (Tylor, 1990). The agreement between the novel and anticipated r_1-value methods was assessed using Cohen's kappa based on a r_1-value cut-off of 0.3. Prevalence-adjusted bias-adjusted kappa (PABAK) was calculated as another index of agreement (Byrt et al., 1993). Bias and repeatability of the novel r_1-value method were assessed using Bland and Altman diagrams (Ludbrook, 2002). Scatter plots and coefficient of variations were used to assess day-to-day variation of the titre for the standardised positive control.

All statistical procedures were performed using IBM SPSS Statistics (Version 25, International Business Machines Corp., Armonk, New York, USA) and results were interpreted at the 5% level of significance.

4.4. Results

Eight FMD reference strains (4 SAT1 and 4 SAT2), 20 SAT1, and 21 SAT2 field isolates were included in the study. Six of the 8 reference strains (3 SAT1 and 3 SAT2), were used for sera standardisation. The majority of viruses (66% SAT1 and 40% SAT2) were isolated between 2001 and 2010. The largest number of isolates were from cattle, African buffalo and impala in South Africa (Table 4.1; Figure 4.1). The SAT reference strains and field isolates clustered according to serotype and revealed distinct genetic variants. Although some SAT1 reference viruses clustered separately from the SAT1 isolates, the majority of isolates formed one group. In contrast, SAT2 viruses were genetically diverse forming several genetic clusters (Figure 4.2).

SAT1 convalescent and vaccinated antibody concentrations were comparable with an average $\log_{10}$ titre for the reference viruses of 2.68 and 2.67 respectively. SAT2 vaccinated animal pooled sera had lower $\log_{10}$ antibody titres compared to the convalescent sera pool. However, the $\log_{10}$ VNT titres for the reference virus assumed to be a vaccine strain (ZIM/07/83) were similar between the two sera pools (Table 4.2; Table 4.3). Therefore, sera pools for SAT1 and SAT2 serotypes were not diluted prior to the vaccine matching analysis.

There was a strong positive correlation between the novel and anticipated r_1-values for SAT1 viruses (Spearman rho = 0.84). The positive correlation was even stronger between the novel and anticipated r_1-values for SAT2 viruses (Spearman rho = 0.90) (Figure 4.3 (a, b); Table 4.4 & 4.5). The axis values for Figure 4.3 (a,b) were standardised to provide visual comparison between the two figures The distribution of novel r_1-values for SAT1 field viruses was more variable within the Bland-Altman plot (Figure 4.4 (a)). The mean

difference between novel SAT1 r_1-values and the validation criterion of the Bland-Altman plot was also substantially different from zero. In contrast, the mean differences between r_1-value for SAT2 viruses were close to zero and the distribution within the Bland-Altman plot was less variable (Figure 4.4 (b)). The agreement (kappa) between novel and anticipated r_1-values at the 0.3 cut-off were 0.64 (95% CI, 0.34-0.94) and 0.47 (95% CI, 0.00-1.00) for SAT1 and SAT2 viruses respectively. The agreement estimated by PABAK between the two methods was 0.67 and 0.84 for SAT1 and SAT2 viruses, respectively. There was no apparent time trend within the standardized positive control titres and the coefficient of variation over time (different testing days) was less than 6% for both serotypes (Figure 4.5 (a, b); Table 4.5).

Table 4.1: Description of the FMD viruses included in the study of a novel r_1-value calculation technique

		SAT1 *(n=24)*	**SAT2** *(n=25)*
Isolation years	1980 - 1990[1]	**1** (04%)	**3** (12%)
	1991 - 1995	**3** (13%)	**4** (16%)
	1996 - 2000	**4** (17%)	**2** (08%)
	2001 - 2005	**8** (33%)	**4** (16%)
	2006 - 2010	**8** (33%)	**6** (24%)
	2011 - 2015	**0** (00%)	**6** (24%)
Species	Cattle	**7** (29%)	**9** (36%)
	African buffalo	**16** (67%)	**13** (52%)
	Impala	**1** (04%)	**03** (12%)
Location	South Africa Kruger National Park	**11** (46%)	**12** (48%)
	South Africa FMD protection zone	**11** (46%)	**10** (40%)
	Other southern African countries	**2** (08%)	**3** (12%)
Virus type	Reference strains	**4** (16.6%)	**4** (16%)
	Field isolates	**17** (70.8%)	**18** (72%)
	Other field isolates (challenge strains)	**3** (12.5%)	**3** (12%)

[1]Only reference strains were isolated during this period

Table 4.2: Comparison of antibody titres of vaccinated and convalescent animal sera using the virus neutralization test (VNT) for SAT1 and SAT2 FMD reference viruses from southern Africa

Virus	**VNT titres (log_{10}) vaccinated sera**				**VNT titres (log_{10}) convalescent sera**			
	Read 1	Read 2	Mean	COV	Read 1	Read 2	Mean	COV
SAT1								
BOT/01/06	2.46	2.56	2.51	2.81	2.66	2.46	2.56	5.52
ZAM/01/06	2.83	2.63	2.73	5.18	2.60	2.43	2.52	4.78
KNP/196/91	2.84	2.71	2.78	3.31	2.86	3.03	2.95	4.08
Mean	**2.71**	**2.63**	**2.67**	**N/A**	**2.71**	**2.64**	**2.68**	**N/A**
SAT2								
BOT/04/06	1.96	1.76	1.86	7.60	2.76	2.96	2.86	4.95
KNP/19/89	1.80	1.72	1.76	3.21	2.6	2.78	2.69	4.73
ZIM/07/83	2.71	2.67	2.69	1.05	2.81	2.98	2.90	4.15

Table 4.3: Comparison of virus neutralization test (VNT) titres for sera standardisation vs actual vaccine matching test for SAT1 and SAT2 FMD reference viruses from southern Africa

Virus	**VNT titres (log10) vaccinated sera**			**VNT titres (log10) convalescent sera**		
	Sera standardisation (*Mean*)	Vaccine matching *(Mean)*	COV[1]	Sera standardisation (*Mean*)	Vaccine matching *(Mean)*	COV
SAT1						
BOT/01/06	2.51	2.62	2.49	2.56	2.62	1.64
ZAM/01/06	2.73	2.50	6.36	2.52	2.62	2.89
KNP/196/91	2.78	2.07	20.58	2.95	2.62	8.26
Mean						
SAT2						
BOT/04/06	1.86	1.38	21.20	2.86	2.91	1.23
KNP/19/89	1.76	2.01	9.20	2.69	3.31	14.51
ZIM/07/83	2.69	3.09	9.79	2.90	2.86	0.86

[1]COV: coefficient of variation.

Table 4.4: Virus neutralization test (VNT) titres, coefficient of variation and r_1-values for SAT1 FMDV isolates from southern Africa (1991-2015)

Type	Virus	Isolation year	Log_{10} average titres (commercial vaccine)	COV[1] %	Log_{10} average titres (positive control)	COV %	Anticipated r_1-value	Novel r_1-value
Reference strains	BOT/01/06[2]	2006	2.60	00.00	2.62	16.33	1.00	0.96
	ZAM/01/06	2006	2.50	13.37	2.62	16.33	0.78	0.75
	SAR/09/81	1981	2.11	13.00	2.82	03.66	0.32	0.19
	KNP/196/91	1991	2.07	00.00	2.62	16.33	0.30	0.28
Challenge strains	SAR/10/10	2010	2.77	11.92	2.82	03.66	1.48	0.89
	SAR/21/10	2010	2.46	32.12	2.87	11.27	0.72	0.39
	SAR/08/10	2010	2.52	36.80	2.87	11.27	0.82	0.45
Field isolates	SAR/33/00	2000	2.55	05.33	2.77	11.27	0.89	0.61
	KNP/22/08	2008	2.20	23.39	2.77	11.27	0.40	0.27
	KNP/11/03	2003	2.22	00.00	2.62	16.33	0.41	0.40
	KNP/01/01	2001	2.34	03.36	2.61	16.25	0.55	0.54
	KNP/17/96	1996	1.95	NA[3]	2.61	16.25	0.22	0.22
	KNP/41/95	1995	1.86	00.00	2.61	16.25	0.18	0.18
	SAR/07/03	2003	2.63	00.00	3.09	06.76	1.07	0.35
	SAR/01/00	2000	2.01	16.64	2.61	16.25	0.25	0.25
	KNP/03/02	2002	2.61	09.36	2.61	16.25	1.00	0.99
	SAR/08/02	2002	2.03	05.24	2.82	03.66	0.27	0.16
	SAR/02/09	2009	2.45	14.70	2.82	03.66	0.71	0.43
	KNP/03/03	2003	2.00	22.48	3.04	10.87	0.25	0.09
	SAR/09/03	2003	2.80	10.90	3.04	10.87	1.59	0.58
	KNP/45/92	1992	2.51	01.43	3.04	10.87	0.80	0.29
	KNP/397/98	1998	2.10	16.64	3.04	10.87	0.32	0.12
	KNP/10/03	2003	2.99	04.29	3.06	32.12	2.44	0.85
	SAR/02/10	2010	2.44	12.02	2.87	11.27	0.69	0.37

[1] COV: coefficient of variation.

[2] Anticipated SAT1 virus to be included in the trivalent commercial vaccine.

[3] The duplicate sample had no reading; therefore, this result is a single reading.

Table 4.5: Virus neutralization test (VNT) titres, coefficient of variation and r_1-values for SAT2 FMDV isolates from southern Africa (1991-2015)

Type	Virus	Isolation year	Log_{10} average titres (commercial vaccine)	COV[1]%	Log_{10} average titres (positive control)	COV%	Anticipated r_1-value	Novel r_1-value
Reference strains	ZIM/7/83[2]	1983	3.09	05.44	2.86	00.00	1.00	1.69
	BOT/4/06	2006	1.34	22.50	2.91	00.00	0.02	0.03
	ZIM/14/90	1990	1.95	00.00	2.86	00.00	0.07	0.12
	KNP/19/89	1989	2.01	16.64	3.30	10.43	0.08	0.05
Challenge strains	SAR/4/14	2014	1.65	00.00	3.01	16.26	0.04	0.04
	SAR/1/13	2013	1.92	05.14	2.86	00.00	0.07	0.11
	SAR/15/13	2013	1.81	16.44	3.01	16.26	0.05	0.06
Field isolates	KNP/11/07	2007	2.43	34.46	2.91	00.00	0.22	0.33
	KNP/1/10	2010	1.70	16.64	2.91	00.00	0.04	0.06
	SAR/1/10	2010	2.04	43.44	2.91	00.00	0.09	0.13
	KNP/16/93	1993	2.46	00.00	2.94	09.55	0.23	0.33
	KNP/06/96	1996	1.81	16.44	2.94	09.55	0.05	0.07
	SAR/3/04	2004	1.56	00.00	2.86	00.00	0.03	0.05
	SAR/1/08	2008	1.84	12.30	2.86	00.00	0.06	0.09
	SAR/11/1919	2011	1.65	07.41	3.16	16.29	0.04	0.03
	KNP/9/03	2003	1.91	15.71	3.16	16.29	0.07	0.06
	KNP/5/91	1991	1.41	05.44	3.16	16.29	0.02	0.02
	KNP/1678/98	1998	1.31	17.25	3.16	16.29	0.02	0.01
	KNP/12/08	2008	1.68	16.85	2.99	16.26	0.04	0.05
	KNP/31/95	1995	2.33	08.46	2.99	16.26	0.17	0.22
	SAR/1/03/2	2003	1.65	00.00	2.70	10.42	0.04	0.09
	SAR/12/0050	2012	2.18	17.04	3.14	12.03	0.12	0.11
	KNP/1/11	2011	1.80	32.22	3.14	12.03	0.05	0.05
	KNP/32/92	1992	1.66	31.93	3.14	12.03	0.04	0.03
	SAR/1/01	2001	1.35	03.71	3.15	26.49	0.02	0.02

[1] COV: Coefficient of Variation between duplicates.

[2] Anticipated SAT2 strain to be included in the commercial trivalent vaccine.

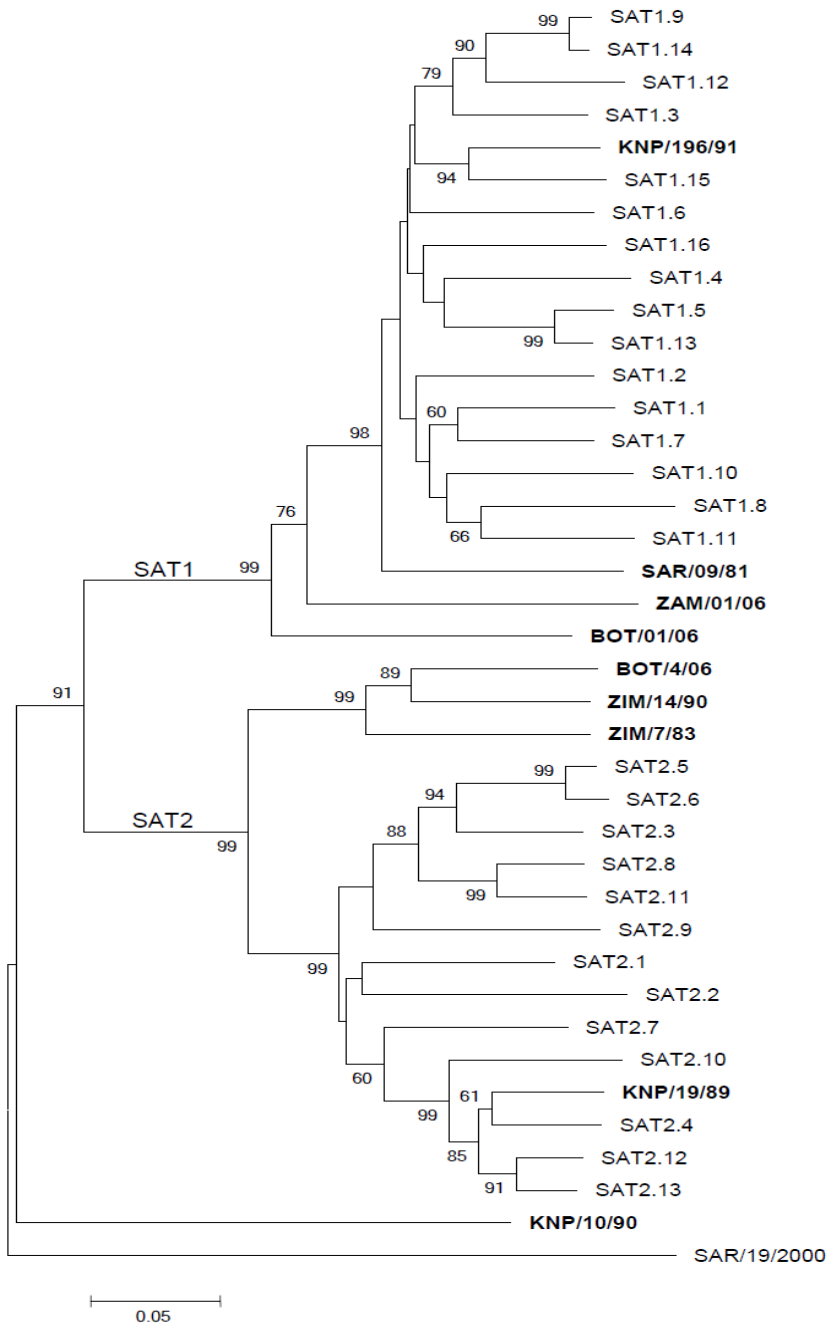

Figure 4.2. A phylogenetic tree depicting the relationships of SAT1 and SAT2 FMD viruses isolated in South Africa between 1991 -2015 based on partial VP1 sequences.

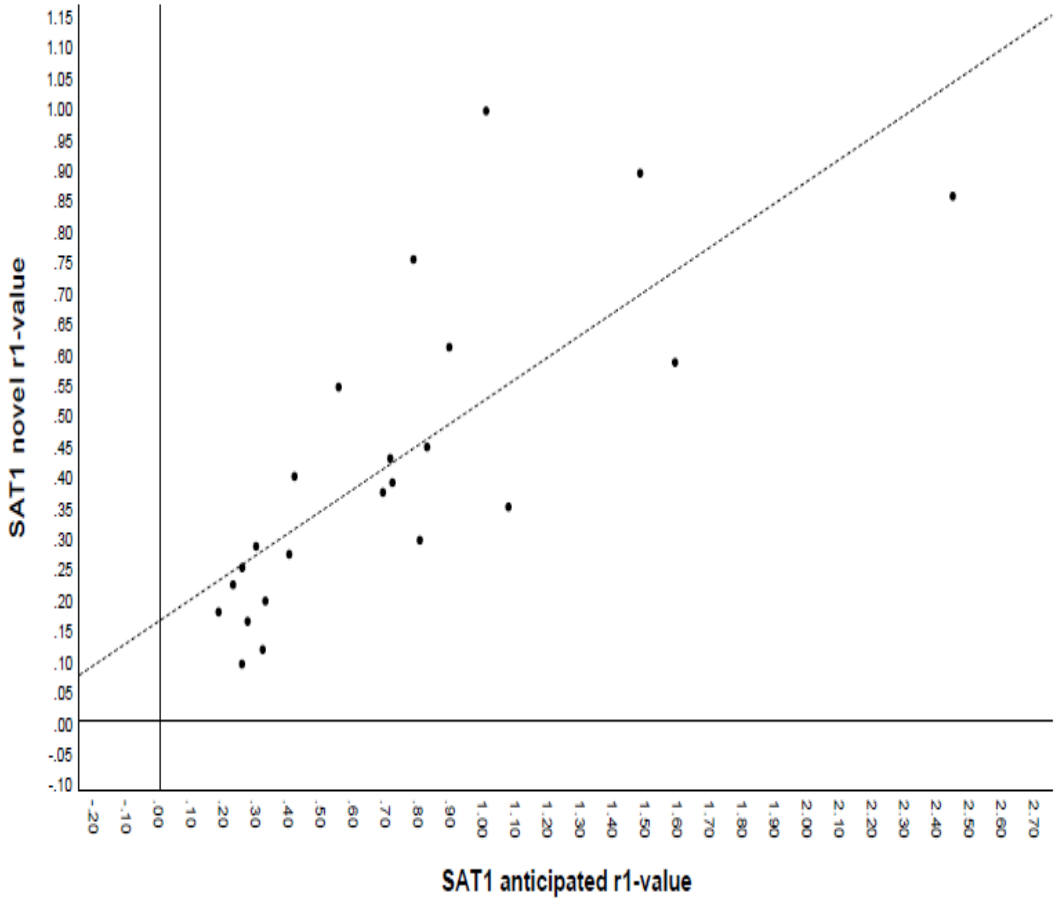

Figure 4.3 (a). Spearman correlation of anticipated and novel r_1-values for SAT1 (Spearman rho = 0.84; P < 0.01) FMD virus isolates from South Africa (1991-2015).

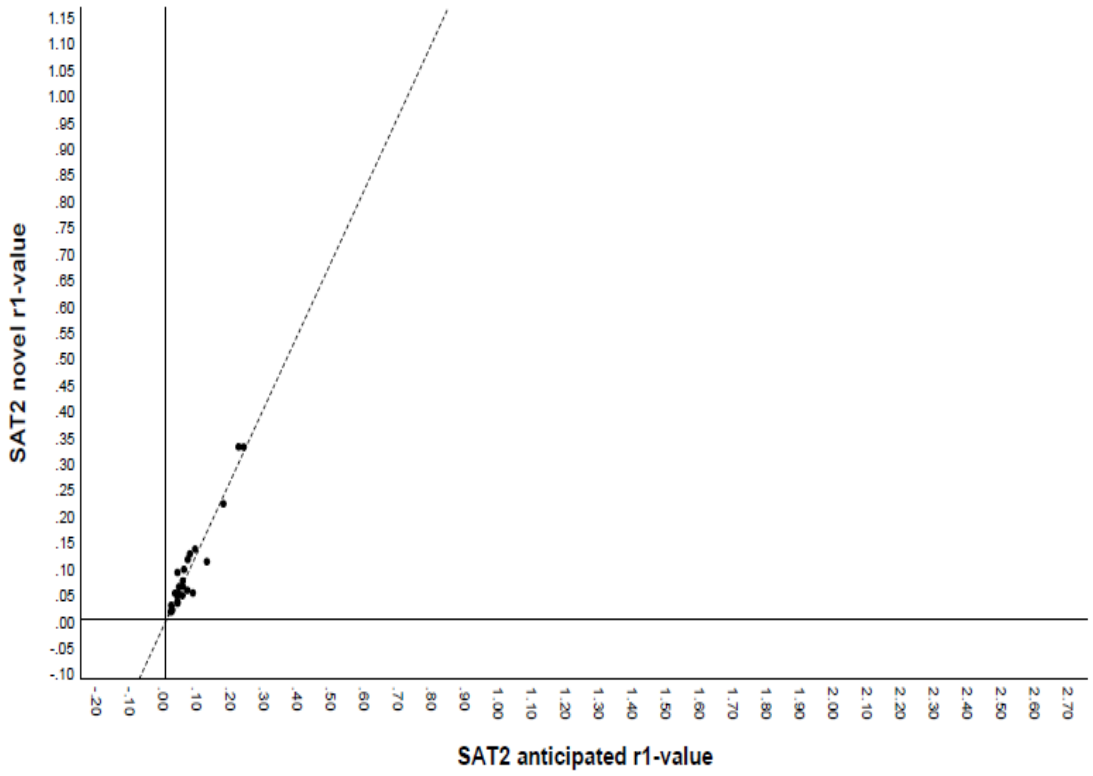

Figure 4.3 (b). Spearman correlation of anticipated and novel r_1-values for SAT2 (Spearman rho = 0.90; P < 0.01) FMD virus isolates from South Africa (1991-2015)

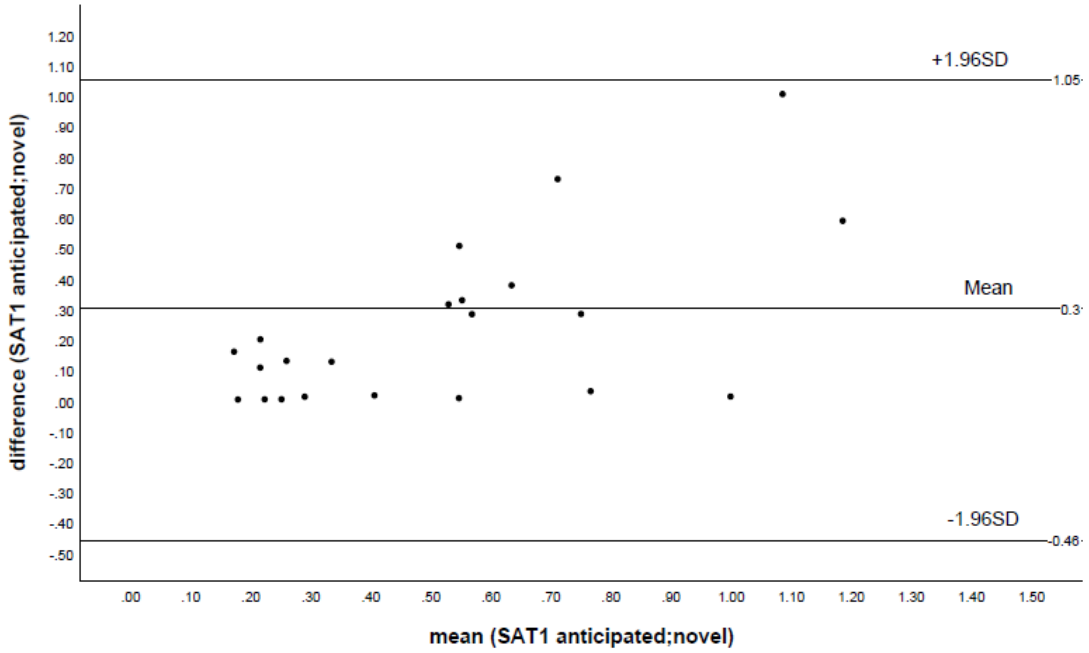

Figure 4.4 (a). Bland-Altman Plot for SAT1 FMDV isolates from South Africa (1991-2015)

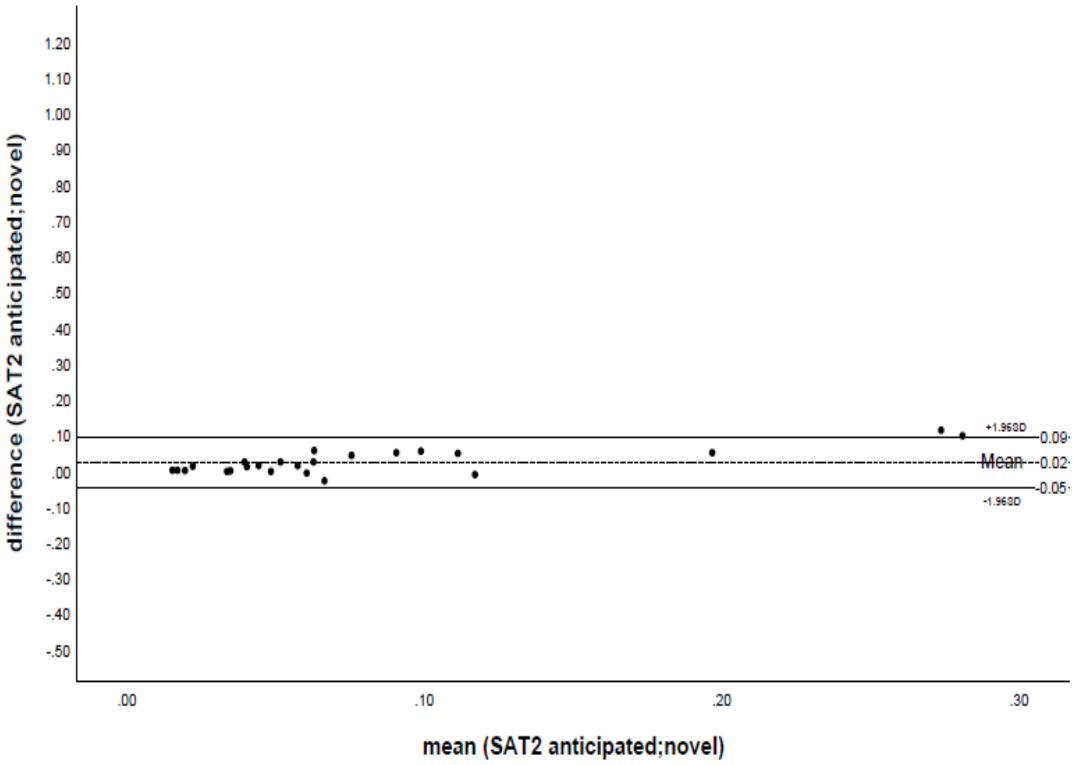

Figure 4.4 (b). Bland-Altman Plot for SAT2 FMDV isolates from South Africa (1991-2015)

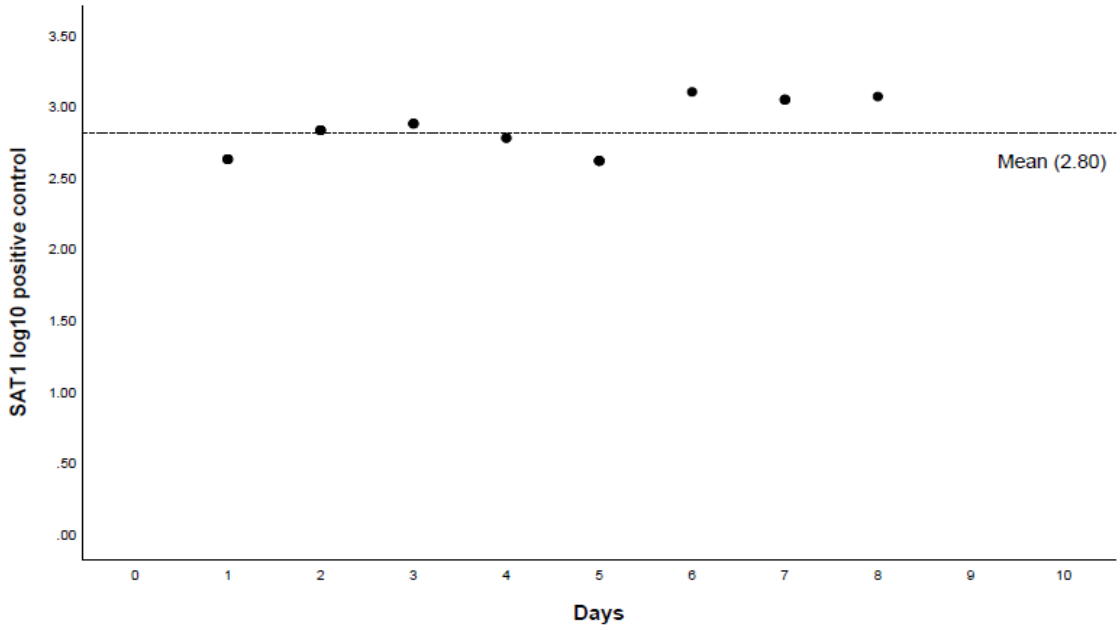

Figure 4.5 (a). Scatter plot for SAT1 FMDV isolates from South Africa (1991-2015) to assess challenge virus against challenge sera (positive control) r1-values day-to-day variation

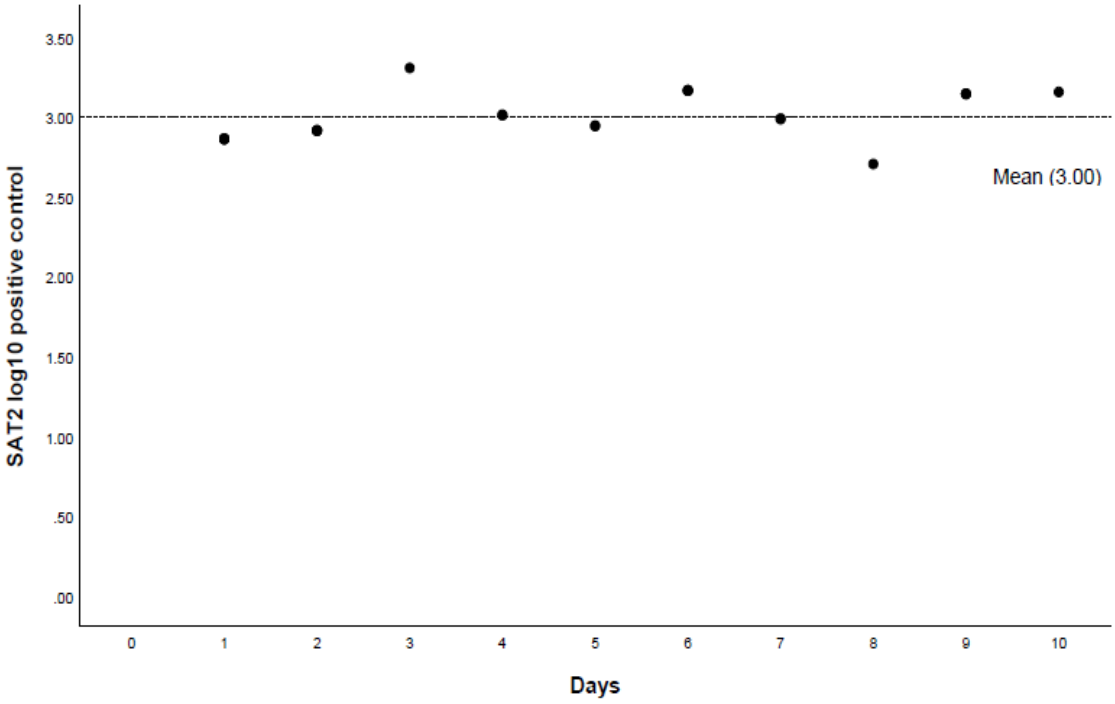

Figure 4.5 (b). Scatter plot for SAT2 FMDV isolates from South Africa (1991-2015) to assess challenge virus against challenge sera (positive control) r_1-values day-to-day variation

4.5. Discussion

Appropriate vaccine strain selection is an important component of FMD control programmes in endemic settings (Doel, 2003). One of the main challenges for disease control is the identification of a vaccine antigen that will protect against currently circulating viruses (Reeve et al., 2010). In the South African FMD Protection Zone with Vaccination, cattle are vaccinated against FMD every four months by South African veterinary services using a trivalent inactivated vaccine containing SAT1, SAT2 and SAT3 antigens (Aftovax®, Merial Animal Health Limited /Botswana Vaccine Institute, Gaborone).

The SAT1 and SAT2 field isolates included in this study represented a diverse group of viruses to account for this expected variability. At least one virus from each of the FMD outbreaks within South Africa during the period 1991-2015 was included in the study. The evaluated viruses included current as well as historical viruses as a broad representation of the FMDV occurring in South Africa. Several SAT1 and SAT2 partial VP1 sequence data for the chosen viruses of this study were previously published (Bastos et al., 2001; Bastos et al., 2003; Vosloo et al., 2007; Phologane et al., 2008; Maree et al., 2011; Brito et al., 2016; Jori et al., 2016; Reeve et al., 2016).

Typical r_1-value calculations require the homologous vaccine virus to be known (Paton et al., 2005). However, vaccine manufacturers might not reveal information regarding the viruses used for commercial vaccine production. The novel r_1-value method was developed as a modification of a sample-to-positive ratio, often employed for the interpretation of ELISA results (Pare et al., 1995; Lanyon et al., 2013). This approach

would be important in situations where it is not possible to obtain the homologous vaccine virus.

The novel r_1-values were strongly correlated to the anticipated r_1-values for SAT1 viruses and very strongly correlated to SAT2 viruses. The SAT1 regression line had a positive y-intercept indicating an overestimation of r_1-values. This suggests a bias of the method within the SAT1 viruses. This likely can be attributed to the virus that was chosen as the assumed homologous virus for r_1-value calculation (BOT/01/06). Although the prediction based on the assumed homologous virus was quite good, it is unlikely that this virus was the true vaccine strain. Evidence of this is that four of the field viruses had r_1-values above 1 (SAR 10/10; SAR 07/03; SAR 09/03; KNP 10/03). In contrast, the novel and anticipated r_1-values calculated for SAT2 viruses were more strongly correlated and there was little evidence of bias in the estimation. The mean difference between novel and anticipated r_1-values was close to zero and there was no obvious pattern suggestive of a systematic error. It therefore appears that the SAT2 virus selected as the assumed homologous virus might be the actual virus in the commercial vaccine (ZIM/07/83). Alternatively, the assumed homologous virus has a very close antigenic relationship to the true vaccine virus. A previous study (Maree et al., 2011) identified the chosen virus as an inactivated vaccine strain used along the borders of South Africa. The unadjusted agreement between the novel and anticipated SAT2 r_1-values may have been biased by the low prevalence of field viruses with a good vaccine match. The SAT2 novel and anticipated r_1-values agreement increased substantially from 47 to 84% when adjusting for prevalence and possible bias. Imprecision in Kappa estimates was evidenced by wide confidence intervals due to few discordant results. PABAK might be similarly imprecise,

but the method only adjusts the point estimate of the agreement and not the corresponding confidence intervals.

Virus neutralization methods typically have high variability caused by differences in cell batches viability, susceptibility and viral variability. Variation in viral doses can introduce variation in serum titres, which might introduce error in r_1-value calculations (Rweyemamu, 1984). The intra and inter assay microneutralization variability is considered acceptable at 18 - 26% and 28 - 30% respectively (Smith and Gilbert, 2017). An acceptable coefficient of variation for day-to-day variation within VNT is 15% (McVicar et al., 1974). The day-to-day variation within the current study was well within the acceptable range for the standardized positive control (challenge virus against challenge sera pool). Therefore, neither day-to-day variation in VNT titres nor improved proficiency in VNT application techniques over time can be the source of the different results between SAT1 and SAT2 viruses.

Fifty-eight percent (14/24) of the evaluated SAT1 viruses (reference and field) had an adequate vaccine match based on the novel r_1-value method. In contrast, only 12% (3/25) of the SAT2 isolates were antigenically similar to the vaccine strain based on the novel r_1-value calculation method. This difference is likely a reflection of the high variability of SAT2 viruses. SAT2 viruses have more VP1 genetic sequence variation compared to other serotypes (Haydon et al., 2001; Brito et al., 2014; Lazarus et al., 2018). A previous study also reported that SAT2 viruses from the region did not have a good antigenic match when tested against the virus we used as the assumed homologous vaccine virus (Maree et al., 2011). A study from Kenya also reported low effectiveness of vaccination for SAT2

FMDV serotypes (Lyons et al., 2015). The extensive antigenic variation of SAT2 viruses has been known for over 30 years (Ndiritu et al., 1983).

The novel r_1-value calculation method must have comparable precision and accuracy to the standard method to be an effective tool for use in vaccine matching. Serum titres obtained by different test systems cannot be compared directly; hence the novel method requires implementation of a rigorous validation protocol employing different FMDV serotypes. Standardisation of the convalescent and vaccinated sera pools is a critical factor when conducting this type of analysis. It might be expected that titres from convalescent animals would be higher than vaccinated animals (Hamblin et al., 1987). Calculated r_1-values can also vary depending on whether or not the sera were pooled prior to testing (McVicar et al., 1974; Brehm et al., 2008). Pooling of serum samples reduces the inter-animal and inter-trial variation, irrespective of the number of serum samples in the pool (ranging from 2 to 16) (Mattion et al., 2009). In the present study, sera were pooled from 50 vaccinated animals per serotype. Convalescent sera from the eight experimentally infected animals were also pooled. Unprotected animals have lower serum titres and low titres are less suitable for r_1-value determination (Mattion et al., 2009). The current study selected sera from animals four to sixteen weeks post-vaccination with high/positive antibody titres of ≥ 2.2 $\log_{10}$ as determined previously by LPBE (Lazarus et al., 2018). The employed methods reduced the likelihood of pooling and low titres influencing results and facilitated the standardisation of vaccinated and challenge sera pools.

4.6. Conclusion

An advantage of the novel r_1-value method is that it makes vaccine matching possible in absence of knowledge concerning the homologous vaccine virus or having the strain available as diagnostic reagents in the laboratory. However, our results require a confirmatory evaluation in a study in which the true homologous virus is known with certainty. These preliminary results support the feasibility, validity and reliability of the new approach. In addition, the presented vaccine matching results are consistent with SAT2 FMDV having high antigenic variability and the low proportion of viruses with a good match is a concern for FMD control in South Africa.

Chapter v

Spatial risk assessment of foot-and-mouth disease occurrence and spread in South Africa (2007-2016)

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5.1. Abstract

Foot-and-mouth disease is a controlled disease in accordance with the South African Animal Diseases Act (Act 35 of 1984) and the country was classified by the OIE as having an FMD free zone without vaccination in 1996. FMD control in South Africa includes animal movement restrictions placed on cloven-hoofed species and products, prophylactic vaccination of cattle, clinical surveillance, and disease control fencing to separate livestock from wildlife reservoirs. The objective of this study was to create spatial risk maps for the FMD protection zone of South Africa.

Eleven risk factors associated with FMD occurrence and spread were used to build a weighted linear combination scores model incorporating FMD expert opinion. Smoothed Bayesian kriged maps were generated for the 11 individual risk factors and overall risk scores for FMD occurrence and spread. Descriptively, vaccine matching was believed to have a great influence on both FMD occurrence and spread. Expert opinion also suggested that FMD occurrence was influenced predominantly by vaccination practices, while cattle populations, cattle inspection (surveillance) and animal movement were important secondary determinants of FMD spread. Highly effective cattle inspections were observed within areas that previously reported FMD outbreaks; indicating the importance of cattle inspection (surveillance) as an imperative element associated with FMD outbreaks detection.

Maintaining an FMD free status without vaccination requires frequent monitoring and high-risk areas for FMD could be used to design targeted surveillance.

Keywords: Bayesian kriging, risk, surveillance

5.2. Introduction

Foot-and-mouth-disease (FMD) is a contagious transboundary animal disease that is considered to be one of the most important animal diseases globally, including within the southern African region (Sinkala et al., 2014). This importance is due to its effects on regional trade in livestock, wildlife and other agricultural products (Grubman and Baxt, 2004). The disease is caused by infection with FMD virus (FMDV), which belongs to the genus *Aphthovirus* within the family *Picornaviridae* (Kitching et al., 2005). There are seven serotypes of FMDV: O, A, C, Asia 1 and Southern African Territories (SAT) 1, 2 and 3 (Larska et al., 2009). Wildlife play an important role in the transmission of FMDV in southern Africa due to African buffalo (*Syncerus caffer*) being a carrier of SAT serotypes and the principal reservoir of infection for domestic livestock (Vosloo et al., 2002; Brahmbhatt et al., 2012).

South Africa controls FMD by separating wildlife from livestock with disease fencing, vaccinating cattle, movement control of cloven-hoofed animals and products and surveillance (DAFF, 2014). The country is FMD free without vaccination; however, South Africa lost its FMD free status in January 2019 due to a SAT2 FMD outbreak outside the protection zone of Limpopo Province and the free status is yet to be reinstated (DAFF, 2019).

Disease risk assessment methods range from visualization to disease modelling (Carroll et al., 2014). A common method used for risk estimation utilizing quantitative data, published literature or expert opinion is a weighted linear combination followed by mapping (Clements et al., 2006; Pfeiffer et al., 2008). Spatial risk mapping is performed to assist in developing risk management policies and strategies (Pfeiffer et al., 2008). Spatial analysis of FMD data can be performed to describe the geographical patterns and

to ultimately understand the epidemiology of disease spread. Furthermore, risk mapping can also be used to visualize the progression of disease epidemics including disease introduction with local or long-distance disease spread. This analytical approach assists in identifying high-risk areas for virus introduction or transmission (Premashthira et al., 2011). Kriging is a well-established spatial geostatistical interpolation technique (Perez et al., 2006; Yasrebi et al., 2009). Empirical Bayesian Kriging (EBK) is an interpolation technique that incorporates the uncertainties related to the plotting of variograms. The method accounts for uncertainty in estimating the semi-variogram by using many semi-variogram models rather than a single one (Krivoruchko, 2012; ESRI, 2019a).

Risk factors associated with FMD introduction and spread include the following: i) disease endemicity status, ii) applied control measures, iii) animal density, iv) type of production systems, v) geographical location, vi) social aspects and vii) environmental factors. Several studies have estimated risk factors for farm-level transmission but, the literature lacks comprehensive studies on FMD risk assessment and mapping in communal farming settings (Bessell et al., 2010a; Bessell et al., 2010b).

In an endemic FMD setting, it has been reported that production system, intermingling of cattle at grazing areas and water sources, movement of infected animals, high herd mobility, presence of a major livestock market, adjacency to a national park, density of small ruminants, drought and cross border movements are risk factors for FMD infection in pastoral systems (Cleland et al., 1996; Megersa et al., 2009; Dukpa et al., 2011; Jemberu et al., 2016; Nyaguthii et al., 2019). Other factors associated with FMD occurrence might include keeping small ruminants, purchasing livestock at markets,

density of cattle herds and close proximity to slaughterhouses (Perez et al., 2004a, b; Lindholm et al., 2007; Megersa et al., 2009).

Roads and proximity to borders play a major role in the endemic and epidemic phases of FMD (Allepuz et al., 2015). The use of a shared bull, the number of animals sourced from other farms, cattle purchases from livestock markets, use of communal dipping and multiple species sharing the same farm are factors associated with FMD introduction (Nyaguthii et al., 2019).

In southern Africa, the risk of FMD introduction and spread is influenced by the presence of African buffalo (*Syncerus caffer*), the wildlife reservoir for the SAT serotypes (Thomson et al., 2003). Contacts between African buffalos and cattle are often close to water points and rivers (Miguel et al., 2017) and fence damage increases the risk of this contact (Mogotsi et al., 2016). Fence maintenance and landscape characteristics are important predictors of FMDV transmission from wildlife to livestock (Dion, 2012).

In South Africa, factors associated with FMD transmission include: i) permeability of the Kruger National Park fence, ii) herd immunity of cattle in the protection zone, iii) African buffalo-cattle contact, iv) human population and v) heterogeneity of the landscape along the FMD protection zone fence line (Jori et al., 2009; Dion, 2011, 2012; Jori and Etter, 2016). Furthermore, stray African buffalo events are affected by fencing type, fence permeability, river crossings, elephant density, African buffalo density, fence maintenance and flooding events (van Schalkwyk et al., 2016).

High-risk areas for FMD occurrence and spread are hypothesized to be different. Therefore, the objective of this study was to apply spatial analytical methods to estimate

a risk of FMD introduction and spread within the FMD protection zone of South Africa (2007-2016).

5.3. Material and methods

5.3.1. Study area

In 1996, the International Committee on FMD of the World Organisation for Animal Health (OIE) endorsed South Africa's FMD free status without vaccination. According to the OIE status, the areas excluded from the free zone were the endemically infected Kruger National Park (KNP) and the FMD protection areas (Bruckner et al., 2002).

FMD control areas are divided into three primary FMD zones: infected, protection and free. The majority of the infected zone is the KNP and adjacent wildlife conservation areas. The KNP and adjacent wildlife reserves are separated from communal farming areas by a fence 1.80 - 2.45 metres in height (Furguson and Jori, 2010). The Ndumo Nature Reserve and the Tembe Elephant Park in KwaZulu-Natal Province have also been considered infected since 2011 (DAFF, 2011).

The protection zone (approximately 480 km long and 10 - 20 km wide) is situated adjacent to the infected zone and falls within the three provinces of Mpumalanga, Limpopo, and KwaZulu Natal (DAFF, 2014). The FMD protection zone is subdivided into two areas: the protection zone with vaccination and the protection zone without vaccination. Cattle within the protection zone with vaccination are inspected for FMD at designated dip-tanks (animal assembly points) every 7 days and small stock (i.e. goats, sheep and pigs) are inspected every 28 days. In this zone, cattle are routinely vaccinated every four months using a commercial trivalent vaccine (containing SAT serotypes 1, 2 & 3) (DAFF, 2014). The protection zone without vaccination is situated to the west and south of the protection

zone with vaccination and all cattle in this area are inspected every 14 days. FMD vaccination is not permitted in the protection zone without vaccination or the free zone.

The study was performed in the FMD protection zone with vaccination (PZV) in the South African provinces of Mpumalanga and Limpopo (Figure 5.1). The FMD PZV of Mpumalanga and Limpopo Provinces includes four (Ehlanzeni North, Ehlanzeni South, Nkomazi and Mbombela) and six local municipalities (Musina, Thulamela, Greater Giyani, Ba-Phalaborwa, Maruleng and Collins Chabane), respectively. These study areas are regarded as the KNP human/wildlife/livestock interface adjacent to the FMD infected zone. The study excluded the PZV of KwaZulu-Natal Province since this is a relatively recently designated protection area (2014) and FMD outbreaks have not been recorded since its establishment.

5.3.2. Data collection and management

The statistical unit of analysis was livestock dip-tanks, which are animal assembly points used for routine inspection and disease control. A dip-tank serves at least one village within an average area of 5 km^2.

The Department of Agriculture and Rural Development, Veterinary Services of both Mpumalanga and Limpopo Provinces provided information on all registered dip-tanks including georeferenced locations, total susceptible animals and animal-specific demographics. Animal demographics were extracted from the state veterinarian monthly disease reports. These reports included information on the total number of cattle per dip-tank at the beginning of each month as well as increases (births and in-movement) and decreases (death, out-movement and slaughter) of the population. The reports also provided information on the date and total number of FMD vaccinations administered to

cattle. The disease inspection and dipping report had the weekly cattle inspection information including the total number of cattle inspected every week. The animal movement permit register was examined to extract data on animal movement in and out of all dip-tank locations. Data were collected for the period April 2007 until March 2016. Vaccination data was extracted for the entire study period (2007 - 2016), while only 2009 data (study mid-point) was used to estimate the cattle population, inspection efficiency and permitted movements.

All reported FMD cases in domestic cattle for the same period in the PZV communal farming areas for both Limpopo and Mpumalanga Provinces of South Africa were identified from the OIE database (WAHIS) (Table 5.1). Coordinates for dip-tanks were converted to the Universal Transverse Mercator (UTM) zone 36S World Geodetic System (WGS) 1984 format and plotted using ArcGIS version 10.4 (ESRI, Redlands, California, USA).

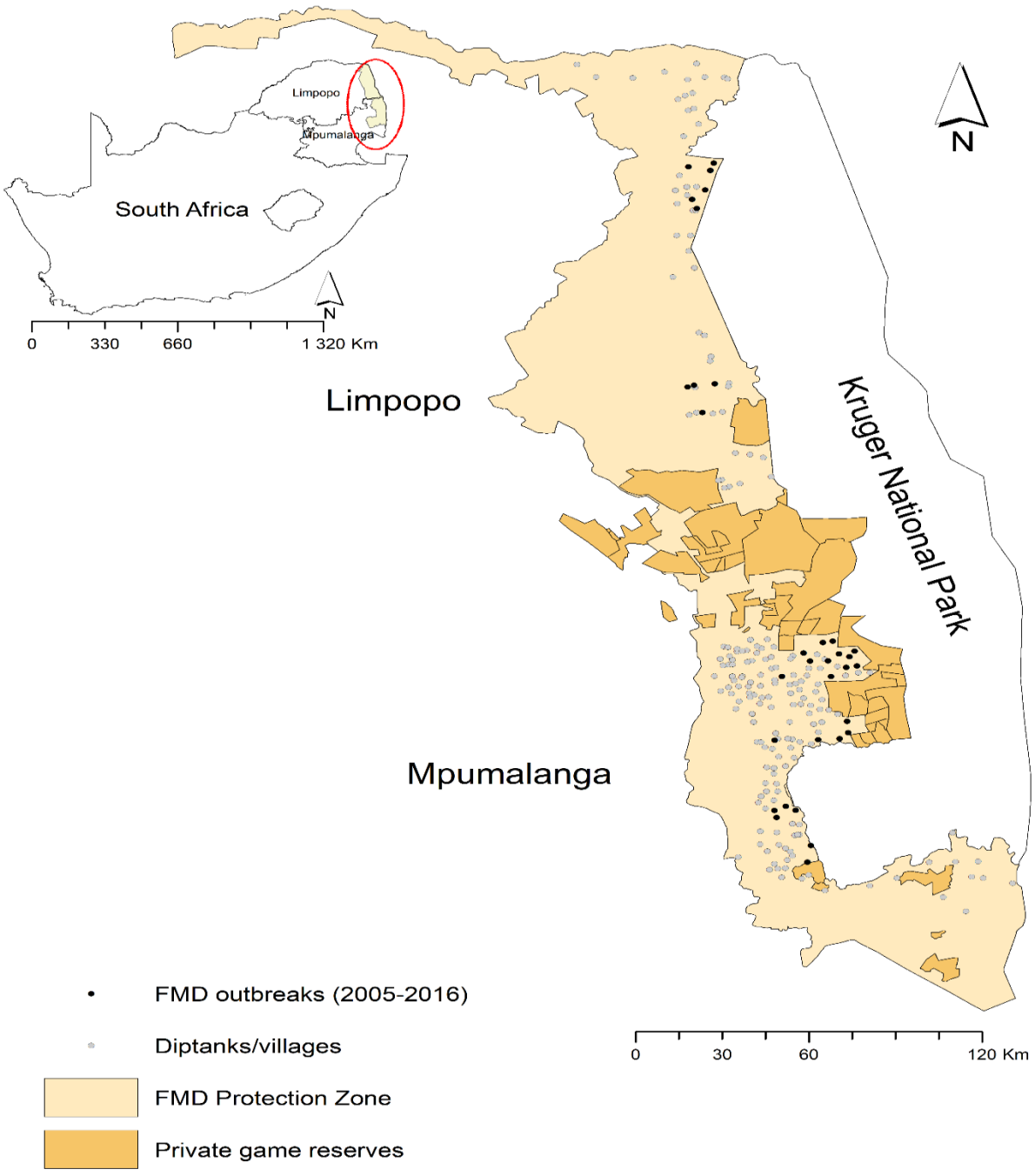

Figure 5.1: South Africa's Limpopo and Mpumalanga Provinces' FMD control zones (infected and protection), livestock dip-tanks and FMD outbreaks 2005-2016 (Black dots are villages/dip tanks that experienced an outbreak during the study whereas the lighter dots did not experience an FMD outbreak).

5.3.3 FMD risk factors

A total of 11 potential risk factors for FMD occurrence and spread in the PZV of South Africa were considered (Table 5.2 and 5.3). These factors were adopted based on the literature and expert opinion. Data concerning cattle population, cattle vaccination numbers, cattle vaccination intervals, cattle inspections, cattle movement into a dip-tank (or village) and cattle movement outside to another dip-tank (or village) were extracted from the veterinary services reports for the year 2009 (study mid-point).

The distance from each dip-tank to the nearest fence of a wildlife reserve, road network and river were estimated using the measuring tool in GIS software (ESRI, Redlands, CA, USA). Human population densities were extracted from the national Statistics South Africa database (2011). Vaccine matching results assigned to each dip-tank were interpolated using data from a previous study (Sirdar et al., 2019) and the zonal statistics tool in the GIS software. A weighted average vaccine match for a combined SAT1 and SAT2 FMD results was calculated by the formula:

$$\frac{\dot{S}SAT1 + \dot{S}SAT2}{Total\ number\ of\ affected\ SAT1\ \&\ SAT2\ diptanks}$$

$$\dot{S}SAT1 = (SAT1\ vaccine\ matching\ for\ each\ diptank)\ x\ (total\ number\ of\ SAT1\ affected\ diptanks)$$

$$\dot{S}SAT2 = (SAT2\ vaccine\ matching\ for\ each\ diptank)\ x\ (total\ number\ of\ SAT2\ affected\ diptanks)$$

Data for each evaluated risk factor were described by calculating the mean, standard deviation, median and interquartile range. Data were compared between Limpopo and Mpumalanga provinces using a Mann-Whitney U test. Statistical analysis was performed using SPSS 26.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and results were interpreted at $p < 0.05$. Spatial risk assessment required a complete data set and missing data were imputed by calculating the mean for each risk factor variable independently by province.

Table 5.1: FMD outbreaks within the South Africa FMD protection zone with vaccination (2005-2016)

Outbreak ID	Province	Duration (months)	Start of outbreak	End of outbreak	Dip-tanks affected (n)	Serotype (SAT[a])	Total susceptible animals	Total cases	Proportion of affected animals
1	Limpopo	5	07/2006	11/2006	2	3	1300	42	0.03
2	Mpumalanga	9	09/2009	05/2010	4	1	9505	757	0.08
3	Mpumalanga	8	12/2011	07/2012	2	2	5510	38	0.007
4	Mpumalanga	4	04/2012	07/2012	5	2	1750	16	0.009
5	Limpopo	6	07/2013	12/2013	1	1[b]	1141	1	0.0008
6	Mpumalanga	19	08/2013	02/2015	12	2	42903	131	0.003
7	Limpopo	7	12/2015	06/2016	5	3	6060	55	0.009
					31		68169	1040	0.015

[a]Southern African Territories.

[b]This outbreak affected other dip-tanks located in the FMD protection zone without vaccination (not part of the study area).

Table 5.2: Risk factors associated with SAT1 and SAT2 foot-and-mouth disease (FMD) ***occurrence*** in the protection zone with vaccination of South Africa.

Potential risk factor for SAT1 and SAT2 FMD occurrence	**Associated hypothesis**	**Indicator measurement**
Cattle population	Increasing cattle density increases the likelihood of FMD outbreak occurrence.	Total number of cattle registered per dip-tank
Proximity to a game reserve	Shorter distance to a game reserve fence increases the likelihood of FMD outbreak occurrence.	Euclidian distance (Km) from each dip-tank to the nearest private or public game reserve
Human population (density)	Higher human density increases the likelihood of FMD outbreak occurrence.	Total number of people per Km^2
Proximity to a road network	Closer proximity to a road network increases the likelihood of FMD outbreak occurrence.	Euclidian distance (Km) from each dip-tank to the nearest road
Proximity to rivers	Closer proximity to rivers increases the likelihood of FMD outbreak occurrence.	Euclidian distance (Km) from each dip-tank to the nearest river
SAT1 vaccine matching	Poorer vaccine matching increases the likelihood of FMD outbreak occurrence.	SAT1 zonal statistic output for each dip-tank generated from 21 SAT1 isolates vaccine matching results
SAT2 vaccine matching	Poorer vaccine matching increases the likelihood of FMD outbreak occurrence	SAT2 zonal statistic output for each dip-tank generated from 20 SAT2 isolates vaccine matching results
Dip-tanks weighted average for a combined SAT1 and SAT2 FMD vaccine matching	Poorer vaccine matching increases the likelihood of FMD outbreak occurrence	((SAT1 vaccine matching for each dip-tank multiplied by (total number of SAT1 affected dip-tanks) + (SAT2 vaccine matching for each dip-tank multiplied by (total number of SAT2 affected dip-tanks)) ÷ total number of affected SAT1 & SAT2 dip-tanks))
Vaccination coverage (vaccination proportion)	Lower vaccination coverage increases the likelihood of FMD outbreak occurrence.	Total number of cattle vaccinated at each dip-tank divided by the total number cattle registered per each dip-tank for every fourth months interval
Vaccination interval	Longer vaccination intervals increase the likelihood of FMD outbreak occurrence.	Total average of months between each vaccination during the study period of 108 months
Cattle inspection (proportion)	Lower inspection effectiveness increases the likelihood of FMD outbreak occurrence.	Total monthly cattle inspected divided by (total weekly inspections per month multiplied by total number of cattle)
Permitted cattle movement into a village/location	Higher number of cattle movements into a village increases the likelihood of FMD outbreak occurrence.	Average monthly permitted movement of cattle into a village/location
Permitted cattle movement outside a village/location	Higher number of cattle movements leaving a village increases the likelihood of FMD outbreak occurrence (within the village sending the cattle out).	Average monthly permitted movement of cattle outside to another village/location

Table 5.3: Risk factors associated with SAT1 and SAT2 Foot-and-mouth disease (FMD) ***spread*** in the protection zone with vaccination of South Africa

Potential risk factor for SAT1 and SAT2 FMD Spread	**Associated hypothesis**	**Indicator measurement**
Cattle population	Increasing cattle density increases the likelihood of local FMD outbreak spread.	Total number of cattle registered per dip-tank
Proximity to a game reserve	Shorter distance to a game reserve fence increases the likelihood of FMD outbreak spread.	Euclidian distance (Km) from each dip-tank to the nearest private or public game reserve
Human population (density)	Higher human density increases the likelihood of FMD outbreak spread.	Total number of people per Km^2
Proximity to a road network	Closer proximity to a road network increases the likelihood of FMD outbreak spread.	Euclidian distance (Km) from each dip-tank to the nearest road
Proximity to rivers	Closer proximity to rivers increases the likelihood of FMD outbreak spread.	Euclidian distance (Km) from each dip-tank to the nearest river
SAT1 vaccine matching	Poorer vaccine matching increases the likelihood of FMD outbreak spread.	SAT1 zonal statistic output for each dip-tank generated from 21 SAT1 isolates vaccine matching results
SAT2 vaccine matching	Poorer vaccine matching increases the likelihood of FMD outbreak spread.	SAT2 zonal statistic output for each dip-tank generated from 20 SAT2 isolates vaccine matching results
Dip-tanks weighted average for a combined SAT1 and SAT2 FMD vaccine matching	Poorer vaccine matching increases the likelihood of FMD outbreak spread.	((SAT1 vaccine matching for each dip-tank multiplied by (total number of SAT1 affected dip-tanks) + (SAT2 vaccine matching for each dip-tank multiplied by (total number of SAT2 affected dip-tanks)) ÷ total number of affected SAT1 & SAT2 dip-tanks))
Vaccination coverage (vaccination proportion)	Lower vaccination coverage increases the likelihood of FMD outbreak spread.	Total number of cattle vaccinated at each dip-tank divided by the total number cattle registered per each dip-tank for every fourth months interval
Vaccination interval	Longer vaccination intervals increase the likelihood of FMD outbreak spread.	Total average of months between each vaccination during the study period of 108 months
Cattle inspection (proportion)	Lower inspection effectiveness increases the likelihood of FMD outbreak spread.	Total monthly cattle inspected divided by (total weekly inspections per month multiplied by total number of cattle)
Permitted cattle movement into a village/location	Higher number of cattle movements into a village increases the likelihood of FMD outbreak spread (from the receiving village to a new village).	Average monthly permitted movement of cattle into a village/location
Permitted cattle movement outside a village/location	Higher number of cattle movements leaving a village increases the likelihood of FMD outbreak spread (to a new village).	Average monthly permitted movement of cattle outside to another village/location

5.3.4. Expert opinion elicitation

All evaluated risk factors were weighted ('*risk factor's weight*') using a pairwise comparison method, where each factor was rated according to its relationship to all other factors (Saaty, 1980). Weights were calculated for each factor based on their pairwise rating and assigned a score using the preference response; risk factor *y* could be considered equally to extremely more or (less) important when compared to risk factor *z* in relation to occurrence or spread of FMD (Table 5.4).

Table 5.4: Preference responses and values used for pairwise comparison

Description	**Value**
Extremely more important	16:01
Very more important	08:01
Strongly more important	04:01
Moderately more important	02:01
Equivalent	01:01
Moderately less important	01:02
Strongly less important	01:04
Very less important	01:08
Extremely less important	01:16

The weightings for each risk factor formed a '*comparison matrix*' using single values derived from the distributions of pairwise comparisons. The resulting matrix was reciprocal, so that the pairwise-comparison for risk factor *y* and risk factor *z* was, $a_{yz} = a_{yz}^{-1}$ and all its diagonal elements were similar ($a_{yz} = 1$ *when* $y = z$). The means from the pairwise

'comparison matrix' were calculated to assign each risk factor its relative importance (*'risk factor weight'*) (Boroushaki and Malczewski, 2008; de Glanville et al., 2014).

Pairwise comparisons were conducted by the author, main supervisor, co-supervisor and two co-authors based on their own expert assessment of the evidence available in the literature and their personal experience (de Glanville et al., 2014). All assessors had previously authored peer-reviewed articles related to FMD epidemiology in southern Africa. Consistency of the pairwise '*risk factor's weight'* of the expert's responses were assessed using Pearson correlation coefficient for both the individual expert responses as well as the overall combined responses (simple average). Analyses were performed for FMD occurrence and spread independently.

5.3.5. Spatial interpolation

Data for all risk factors were standardised ('*standardized score*') by subtracting the mean and dividing by the standard deviation. A similar standardisation was performed on the weights elicited from the experts (*'standardized weight'*). The '*standardised weight'* for each risk factor was multiplied by the '*risk factor's weight'* forming a '*weighted score'*. The sum of '*weighted score'* for each dip-tank was calculated and used to generate risk maps using empirical Bayesian kriging (EBK) (Krivoruchko, 2012; Samsonova et al., 2017; ESRI, 2019a). All maps were produced in ArcGIS software version 10.4 (ESRI, Redlands, CA, USA). Spatial risk maps were descriptively validated by projecting the locations of FMD outbreaks during the study period in relationship to the maps (Craig et al., 1999).

5.4. Results

There were a total of 223 dip-tanks within the PZV during the study period (2007-2016). Mpumalanga Province had 168 dip-tanks and Limpopo 55 dip-tanks. Collected data concerning risk factors varied between Limpopo and Mpumalanga Provinces ($p < 0.05$) for all factors except human population density, proximity to a game reserve fence and cattle movement into a village or dip-tank (Table 5.5). Inverse '*risk factor's weight*' responses within experts were strongly correlated for all participants excluding Expert (2) (Table 5.6) (Appendix 3).

The spatial distribution of cattle population was not uniform with higher cattle numbers in the central and northern areas of Limpopo Province. On the other hand, the northern part of Mpumalanga had more cattle numbers compared to the rest of the province (Appendix C-4). Almost all dip-tanks were in close proximity to game reserves and major road networks, except for an area in central Limpopo (Appendix C-5 and Appendix C-6). Distance to rivers was descriptively higher in northern Mpumalanga compared to the rest of the study area (Appendix C-7). Mpumalanga had a slightly poorer SAT1 vaccine match compared to Limpopo (Appendix C-8). The SAT2 vaccine match was inadequate over the entire study area (Appendix C-9) and a similar trend was observed for the weighted vaccine match results (Appendix C-10). FMD vaccination proportion (Appendix C-11) and vaccination intervals (Appendix C-12) were lower in Limpopo Province relative to Mpumalanga. Fewer cattle inspections were performed in the northern areas of Limpopo Province and scattered areas of Mpumalanga (Appendix C-13). Permitted cattle movement into villages was uniformly distributed across the study area with an exception of central Mpumalanga that had higher *in* movements (Appendix C-14). There was also a high

number of *out* movements in an area of southern Mpumalanga (Appendix C-15). Human density was high in the southern western areas of Mpumalanga (Appendix C-16).

The far north of Limpopo Province and the central areas of Mpumalanga were at higher predicted risk of SAT1 and SAT2 FMD outbreak occurrence (Figure 5.2). In contrast, the central areas of Limpopo and the southern parts of Mpumalanga were at higher predicted risk for FMDV spread (Figure 5.3).

Table 5.5: Descriptive statistics for potential risk factors[1] for FMD ***occurrence*** and ***spread*** in the FMD protection zone with vaccination of South Africa (2007-2016)

Potential risk factors (FMD occurrence and spread)							
	Combined study area		**Limpopo Province**		**Mpumalanga Province**		**Mann-Whitney Between provinces**
	Mean (SD)	**Median (IQR)[2]**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**p-value**
Vaccination interval (months)	9.99 (10.59)	5.46 (2.86; 16.00)	20.99 (8.51)	18.35 (15.37; 33.01)	7.39 (9.30)	3.88 (2.57; 6.90)	< 0.001
Vaccination coverage (months)	0.10 (0.07)	0.10 (0.03; 0.16)	0.02 (0.03)	0.02 (0.01; 0.03)	0.13 (0.06)	0.13 (0.07; 0.18)	< 0.001
SAT1 vaccine matching	0.38 (0.06)	0.35 (0.33; 0.37)	0.46 (0.05)	0.47 (0.39; 0.50)	0.36 (0.02)	0.33 (0.32; 0.35)	< 0.001
SAT2 vaccine matching	0.06 (0.03)	0.05 (0.04; 0.08)	0.09 (0.03)	0.09 (0.07; 0.11)	0.05 (0.02)	0.04 (0.03; 0.06)	< 0.001
Dip-tanks weighted average (SAT1 & SAT2 vaccine matching)	0.12 (0.04)	0.11 (0.10; 0.14)	0.17 (0.03)	0.17 (0.13; 0.19)	0.1 (0.03)	0.1 (0.09; 0.12)	< 0.001
Cattle population	647 (383)	592 (366; 871)	869 (488)	939 (367; 1287)	589 (328)	590 (363; 758)	0.002
Cattle inspection (%)	0.43 (0.27)	0.45 (0.28; 0.57)	0.29 (0.48)	0.20 (0.01; 0.30)	0.47 (0.16)	0.48 (0.34; 0.58)	< 0.001
Permitted cattle movement into a village/location	3.59 (8.16)	1.67 (0.83; 3.46)	4.41 (13.50)	1.54 (0.46; 3.01)	3.37 (6.10)	1.67 (0.83; 3.67)	0.222
Permitted cattle movement outside a village/location	5.37 (9.59)	2.25 (0.96; 5.38)	10.56 (13.40)	7.70 (3.80; 11.80)	3.78 (7.48)	1.67 (0.79; 3.08)	< 0.014
Human population (density)	1300 (1116)	1026 (468; 1866)	1055 (735)	1000 (389; 1506)	1371 (1196)	1033 (491; 1911)	0.275
Proximity to a game reserve (km)	8.60 (7.20)	7.08 (3.55; 11.26)	8.80 (8.90)	6.80 (3.70; 10.40)	8.53 (6.58)	7.17 (3.2; 11.83)	0.795
Proximity to a road network (km)	9.65 (10.27)	5.6 (2.25; 13.51)	14 (14.21)	6.98 (3.19; 23.58)	8.22 (8.16)	5.12 (2.03; 12.15)	< 0.013
Proximity to rivers (km)	16.18 (11.49)	13.88 (5.47; 26.08)	7.27 (5.76)	6.17 (2.40; 10.79)	19.1 (11.4)	20.14(8.17; 28.6)	< 0.001

[1]Mean and median values of the absolute numbers of the potential risk factors for FMD occurrence and spread

[2]IQR: Interquartile Range (25th and 75th percentile)

Table 5.6: Internal consistency of the experts' pairwise (inverse) risk factor weighting responses for FMD occurrence and spread

Response	**Person correlation coefficient**
FMD occurrence	
Expert (1)	-1.00
Expert (2)	0.67
Expert (3)	-1.00
Expert (4)	-0.98
Expert (5)	-0.93
Combined (mean) response	-0.93
FMD spread	
Expert (1)	-1.00
Expert (2)	0.24
Expert (3)	-1.00
Expert (4)	-0.97
Expert (5)	-0.92
Combined (mean) response	-0.66

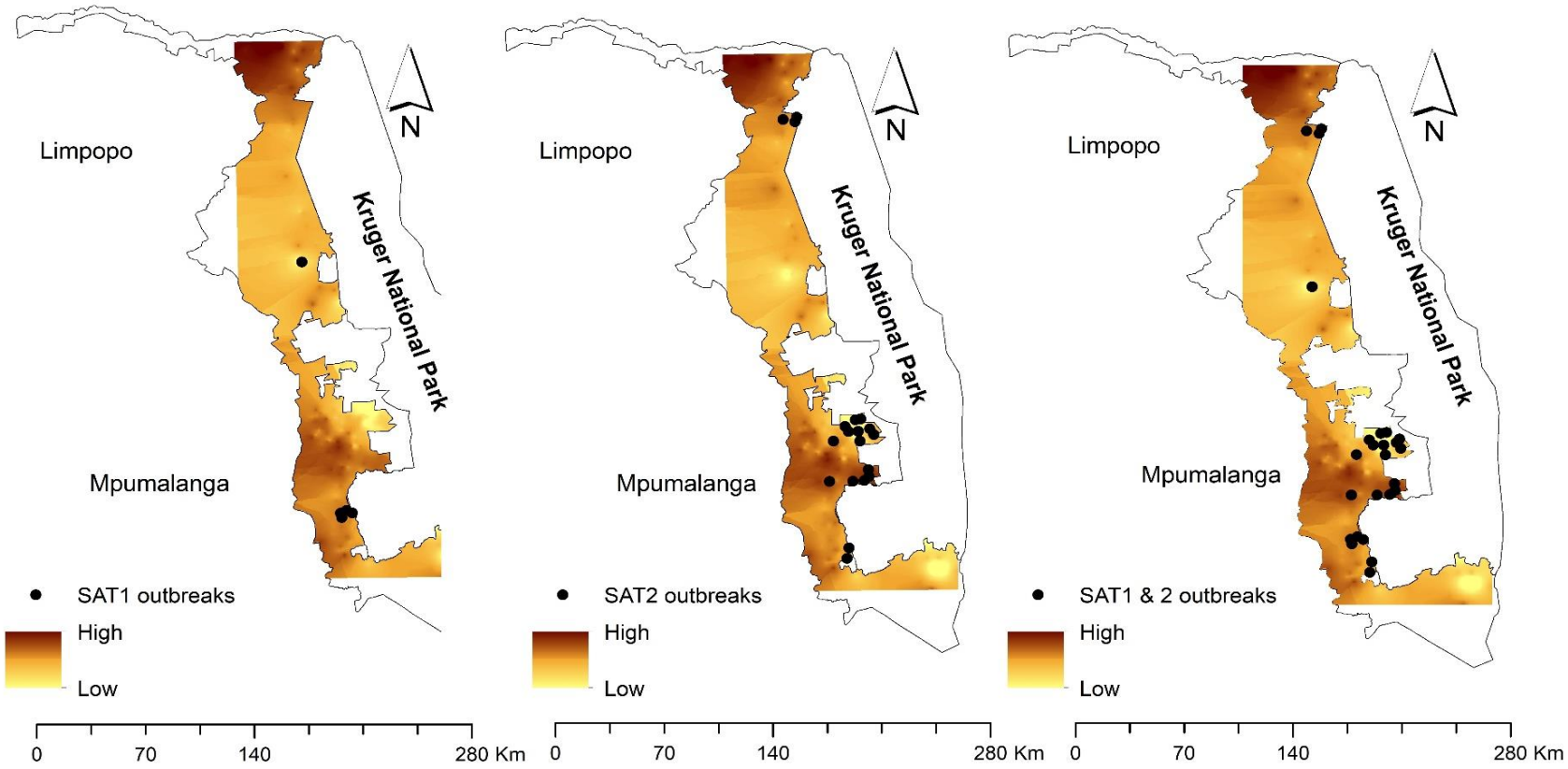

Figure 5.2: (a) Southern African Territories (SAT)1 risk map for FMD ***occurrence*** in the protection zone with vaccination (PZV) with vaccination of South Africa; (b) SAT2 risk map for FMD occurrence in the PZV with vaccination of South Africa; (c) combined SAT1 and SAT2 risk map for FMD occurrence in the PZV with vaccination of South Africa.

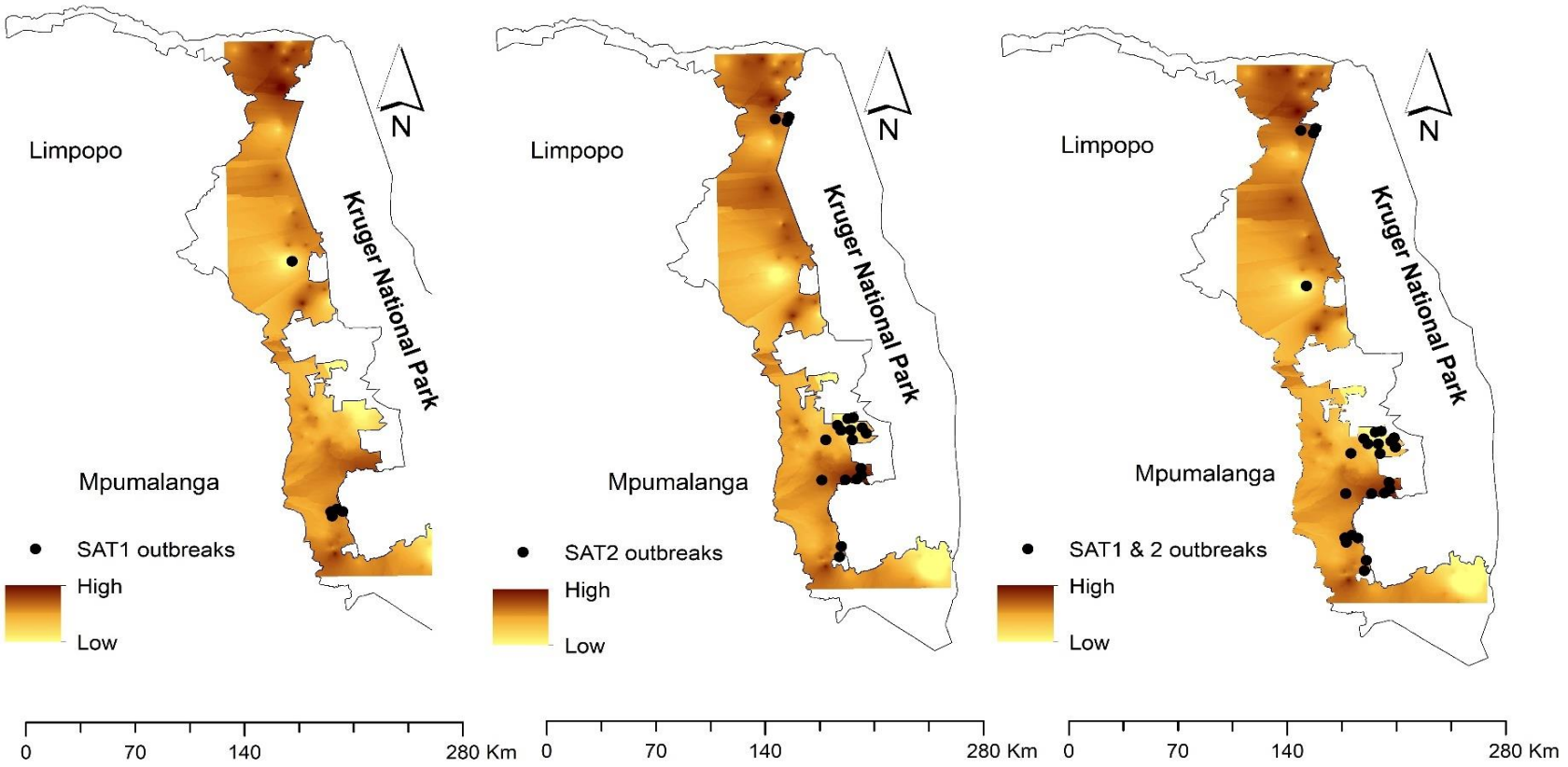

Figure 5.3: (a) Southern African Territories (SAT)1 risk map for FMD ***spread*** in the protection zone with vaccination (PZV) with vaccination of South Africa; (b) SAT2 risk map for FMD spread in the PZV with vaccination of South Africa; (c) combined SAT1 and SAT2 risk map for FMD spread in the PZV with vaccination of South Africa.

5.5. Discussion

Spatial analysis and GIS-based methods have an important role in animal disease investigations (Lawson and Zhou, 2005). Disease introduction and spread should be investigated considering its spatial context (Sanson et al., 1993). This study incorporated risk factor information for FMD occurrence and spread in the PZV of South Africa to determine areas where FMD is more likely to occur and subsequently spread to other locations. The study generated smoothed risk maps with the aim of identifying high-risk areas for applying improved control measures. It is believed that FMD endemic countries often lack the resources necessary to sustain FMD control in effort to open lucrative export markets (van Schalkwyk et al., 2011). The alternative is to develop risk-based surveillance and targeted controls through a complete understanding of risk factors and their spatio-temporal distributions. This approach maximizes the use of limited human resources by applying control measures effectively in high-risk areas.

Data for included risk factors often differed significantly between the two study provinces. However, proximity of dip-tanks to game reserves was not different. This was expected as the PZV was formed as a first line buffer to protect the rest of the country from FMD outbreaks caused by contact between wildlife and livestock. There was also no difference between the two provinces regarding cattle movement *into* a village suggesting similar human activities and demand for consumption. The significant differences between the provinces suggests that different approaches and implementation of FMD control is conducted in each province.

Risk factor standardized weightings by participating FMD experts ranked proximity to game reserve as the most important factor for FMD *occurrence* followed by the total cattle

population. The literature supports this opinion as proximity to national parks or wildlife reserves has been reported to be significantly associated with the risk of FMD outbreaks (Bronsvoort et al., 2004; Ayebazibwe et al., 2010; Allepuz et al., 2015; Jemberu et al., 2016).

Based on the expert opinion in this study, cattle population density was the most important risk factor for FMD *spread* followed by vaccination activities (vaccine matching and vaccination coverage and interval). Cattle density has been previously reported to be associated with the FMD dissemination pathway (Santos et al., 2017). Other studies also reported the positive association between cattle densities and high likelihood of FMD spread (Bessell et al., 2010b; Dukpa et al., 2011). However, a study conducted in Tanzania reported that cattle density had a lower effect on FMD transmission than expected possibly due to the confounding effect of animal movements (Allepuz et al., 2015). The contribution of cattle population to FMD occurrence might be linked to cattle incursions into KNP and subsequent contacts with wildlife (Jori et al., 2011).

On the other hand, cattle inspection proportion, movement *out*, proximity to rivers and road networks were ranked the lowest for the risk of FMD occurrence and spread. Availability of roads could contribute to legal and illegal movements of animals, increased likelihood of FMD detection and access to human activities such as markets. In Ethiopia, it was reported that major livestock markets/routes were associated with animal trade movements and were significant risk factors identified for FMD spread (Jemberu et al., 2016). In Tanzania, proximity to road networks was identified as a risk factor but had a limited effect on FMD transmission relative to other factors (Allepuz et al., 2015). Rivers and waterpoints crossing the KNP fence might play a role in FMD occurrence and spread

through cattle-African buffalo contacts. Contacts between cattle and African buffalo are 1.25 times more likely to occur outside the KNP around rivers and water sources compared to locations without water (Jori and Etter, 2016).

The effectiveness of vaccination depends on the match of the vaccine strain to the circulating viruses (Robinson et al., 2016; Sirdar et al., 2019). The vaccine currently used in the PZV is not a good match for circulating strains in South Africa and this reduces the effectiveness of prophylactic vaccinations (Thomson et al., 2013; Sirdar et al., 2019). The SAT1 vaccine match was better than SAT2. However, the inadequate SAT2 vaccine match was even worse in Mpumalanga compared to Limpopo Province. A recent study conducted in Mpumalanga reported that SAT2 antibody responses were better than SAT1 (Lazarus et al., 2018) and a stronger response might be able to overcome some deficiencies in vaccine matching.

Vaccination is the most frequently reported preventive measure for FMD (Nyaguthii et al., 2019). Regular vaccination and adequate vaccine protection minimize the risk of FMD transmission from wildlife to livestock (Jori et al., 2011). It is assumed based on a basic reproduction number of four that at least 75% of cattle should have protective immunity to FMD to achieve adequate protection of the population as a whole (Pay, 1984). In Mpumalanga, the overall seropositivity was previously reported to be less than 75%, thus potentially increasing the risk of FMD outbreaks (Lazarus et al., 2017). The risk of FMDV transmission from African buffalo to cattle can be reduced when cattle are immunized and contacts are limited (Jori and Etter, 2016).

The FMD vaccination proportion was very poor in Limpopo Province relative to Mpumalanga. The latter is known for excellent dipping attendance and facilities (Lazarus

et al., 2017). Human and operational resources in addition to infrastructure deficiencies might have affected the vaccination practices in Limpopo Provinces. Although Mpumalanga had more dip tanks compared to Limpopo, the dip-tanks were close to each other. In Limpopo the dip-tanks were distributed over a larger area. This might also contribute to the observed poorer vaccination practices due to increased travel costs.

Cattle in the PZV are supposed to be vaccinated every four months (DAFF 2014). Vaccination intervals in Limpopo Province were quite large in relation to the recommendations of the veterinary authorities and vaccine manufacturer. Isolated areas in northern Mpumalanga and far south also had relatively longer intervals. The latter is consistent with a reported prolonged vaccination interval in one area of Mpumalanga (Lazarus et al., 2017; Lazarus et al., 2018). Longer intervals and low percentage of vaccinated cattle has also been reported in Zimbabwe, where it had a negative effect on breaking the FMDV transmission cycle (Miguel et al., 2017).

To our knowledge, no previous reports have analysed cattle inspection practices and their role in FMD outbreaks and spread. However, it is assumed that lower inspection effectiveness increases the likelihood of FMD spread due to the missing of FMDV infected animals. Inspection was very good in Mpumalanga Province and moderate in the southern areas of Limpopo. However, the northern part of Limpopo had poor inspection proportions. The areas with the highest inspection proportion in Mpumalanga were the areas that reported previous FMD outbreaks. This finding might indicate that surveillance is more likely linked to FMD detection rather than occurrence. This finding supports the objectives of the South African FMD Veterinary Procedural Notice (VPN). The VPN states that to prevent FMD occurrence and spread, clinical surveillance must be performed in

the PZV by inspecting cattle every seven days and routinely mouthing at least 10 cattle randomly selected from the presented cattle on each inspection day (DAFF, 2014). However, clinical surveillance alone might not be sufficient and should be supported by a routine (not an ad-hoc) laboratory-based surveillance to account for undetected cases (Teifke et al., 2012). This can be attributed to that fact that FMD SAT outbreaks might be underdiagnosed in the field due to frequently mild or subclinical infections (Jori et al., 2009). The cases reported during the study period might not reflect the actual number of outbreaks that had occurred in the field due to inefficiencies in surveillance efforts.

Many communal farmers within the study area depend solely on livestock for their livelihood. Our results suggest that there are more permitted movements of cattle to other locations than cattle been introduced to dip-tanks within the PZV. This might represent the selling of cattle for income generation. The higher animal movement *outside* to other villages in the central and northern areas of Limpopo Province could be due to the large cattle population in these areas. The higher movement (*into villages*) in the southern parts of Mpumalanga Province could be associated with a higher human population densities and greater demands for consumption.

Animal movements are influenced by differences in the price of livestock between different zones (Sinkala et al., 2014). This difference in price and its economic effect might create apathy for FMD movement control measures. Cultural practices and political aspects can also contribute to the failure to observe or enforce animal movement restrictions. It is believed that animals are moved within the PZV for ritual reasons (personal communication). Violent civil unrest and riots related to civil service delivery

have also affected FMD disease control campaigns and animal movements (Mpumalanga Veterinary Services “internal report”, 2012).

The overall model predicted risk of FMD did not differ considerably from *spread*. This finding is influenced by expert opinions’ ranking of risk factors for occurrence and spread. A different group of experts might have produced a different estimate for introduction and spread. Previous studies at the KNP interface suggested the possibility of undetected FMD spread in cattle after the initial wildlife-livestock transmission (van Schalkwyk et al., 2011). The high-risk area for FMD occurrence in the far north are clearly influenced by the low vaccination proportion, longer intervals between vaccinations and poor inspection efficiency. In contrast, the northern part of Mpumalanga Province, despite being well inspected and with good vaccination coverage/interval, was also identified as a predicted high-risk area for FMD occurrence. A possible reason for this finding is the inadequate vaccine match for SAT2 isolates and poor match for SAT1.

The far north and central areas of Limpopo Province were at relatively higher risk for FMD spread. The far north areas had similar results as FMD occurrence. However, the central areas of the province are likely at higher risk of FMD spread due to higher cattle densities, dip-tanks in close proximity to rivers and considerable movement of animals (*outside* to other locations). Also, the southern parts of Mpumalanga are at higher risk of FMD spread where there were larger human populations.

The study outcomes support and complement the findings of a previous qualitative risk assessment performed for the protection zone of South Africa. This previous study reported that the risk of an FMD outbreak in the communal areas was moderate and influenced by i) permeability of the KNP fence, low cattle herd immunity, efficiency of

regulations regarding FMD control measures and unpermitted cattle movements in response to price differences between zones (Jori et al., 2011).

Although few outbreaks occurred during the study period, FMD outbreaks during the study period were used to visually validate our results. Only seven FMD outbreaks were reported during this period. Two SAT1 outbreaks affecting 5 dip-tanks, three SAT2 affecting 19 dip-tanks and two SAT3 reported in 7 dip-tanks (Table 5.1). Although SAT3 outbreaks affected 16% (7/31) of dip-tanks, they were excluded from the analysis. SAT3 FMDV isolates were not available for vaccine matching in the study. Furthermore, most SAT3 isolates banked by the Transboundary Animal Disease Laboratory (TAD) were recovered from wildlife (Dyason, 2010) rather than cattle.

Validation of the risk models demonstrated that the majority of reported outbreaks occurred in areas with relatively low predicted risk for FMD occurrence and spread. This suggests possible bias in the study methods because these areas are characterized by efficient cattle inspection and vaccination practices. A possible explanation is that efficient cattle inspection is the major determinant whether or not an outbreak is detected and subsequently reported. FMD outbreaks in other locations might have been missed because of inefficient cattle inspection. The total number of reported outbreaks has increased 7-fold during the three years following the study period (2017-2019) and totaling 42 outbreaks (OIE, 2020). This 7-fold increase might be due to a general improvement in surveillance efforts where a higher proportion of true incursions are now detected and appropriately reported. It might also represent changes in other risk factors including livestock movements and issues surrounding vaccination.

There was a large difference in data availability between the two provinces and this might have affected the calculated risk scores and the comparison between provinces. Availability of data, especially in Limpopo Province, was one of the challenges of the study. Informative risk assessment requires credible and complete data to provide an informed outcome (Wieland et al., 2015). Therefore, it is recommended that data storage and management be improved in the study area.

The study did not generate estimates of risk, but rather described variations in absolute risk based on the combination of quantitative data with expert opinion. Although, the model seemed to be biased as outbreaks were observed in low risk areas, the outcome of the model highlights the importance of some risk factors including surveillance that could be used for the revision of current FMD control policies. The areas with relatively higher or lower risk of FMD occurrence and spread are influenced by the included expert opinion. Given the relatively small number of experts that participated in the study, there is a necessity to repeat the modeling approach to accommodate more quantitative data and a larger number of experts.

The mid-point study period data (2009) was used for all risk factors except vaccination coverage, vaccination interval, vaccine matching and distance data. This approach might have created some bias due to the 10-year period of the study. Human population demographics might have changed substantially between the beginning and the end of the study period subsequently causing changes in the cattle population and related farming activities. Animal movement is also related to human activities and densities across villages. A severe drought during the second half of the study period might have also influenced human/animal demographics and activities.

The current study cannot provide outbreak predictions but can aid prevention and control measures by describing the trends in the study area. Expert opinion can be a valuable tool in this analysis (Amaral et al., 2016). The responses of one expert concerning risk factor weighting were not consistent and future studies should incorporate formal training of experts prior to the collection of data for analysis. A broader pool of available experts would also be valuable for future studies.

The movement data only included permitted (legal) movement, which might have affected results as illegal movements are likely associated with higher FMDV transmission risks. Legal movement activities do not necessarily provide a true reflection of the actual number of animals moving within the PZV. However, it is difficult to obtain data on illegal movement. Although, access to livestock markets could be used to estimate the true movement in the PZV by comparing animal census data against legal permits and animals presented for sale in livestock markets. However, this approach will also miss informal markets and door-to-door purchases.

The incorporation of other risk factors including fence breakage, fence maintenance, African buffalo distribution, climate, social and cultural factors might have improved the validity of model results. While this study was a retrospective semi-quantitative study, we propose a quantitative predictive model to be developed to allow better insight into FMD risk factors associated with FMD occurrence and spread. Future studies should be complemented with risk mitigation, management and communication (Mogotsi et al., 2016).

5.6. Conclusion

There are differences in the currently applied FMD control systems between Limpopo and Mpumalanga Provinces that need to be addressed. Regaining and maintaining FMD free status requires frequent monitoring and mapping of areas at risk for FMD outbreaks. The study finding indicates the necessity to enhance animal health surveillance at areas identified as high risk for FMD outbreak occurrence and spread. Detecting actual outbreaks and studying the disease trends within the PZV will assist in designing effective control measure. South Africa could enhance its FMD surveillance activities following the guidelines of the PCP-FMD framework by:

- Introducing vaccine potency testing for every batch that is purchased.
- Renovating dip-tank facilities for improved surveillance and vaccination.
- Performing regular audits of the control and surveillance measures to ensure uniform implementation of FMD control measures.
- Introducing risk-based surveillance to minimize cost and manage resources.
- Performing periodic FMD risk assessments that account for the environmental, cultural and epidemiological dynamics of the disease.
- Providing farmers with compensation as incentive to maintain efficient control measures and improve compliance with movement restrictions.
- Introducing mobile slaughtering facilities in the PZV to incentivize farmers affected by the FMD control measures.
- Promoting commodity-based trade to mitigate the risk of introducing FMDV in the free area and subsequently contribute to the micro and macro economies of the country.

Chapter vi

General Discussion and Conclusion

6.1. Introduction

South Africa's foot-and-mouth disease (FMD) control policy has evolved over time since it was first introduced in 1931 by accommodating different control methods, ranging from animal movement restrictions to passive and active disease surveillance (Thomson, 2008). Several remarkable milestones were achieved over the years including the implementation of the Animal Diseases Act 35 of 1984[1]. The Act described FMD controlled areas, a stamping-out strategy during outbreaks, vaccination of livestock and African buffalo (movement and confinement) control (Moerane, 2008). Implemented control measures limited the occurrence of FMD to less than one outbreak per decade and informed the decision of the International Committee on FMD of the World Organisation for Animal Health(OIE) to endorse South Africa's FMD free status without vaccination in 1996. According to the OIE status, the areas excluded from the free zone were the endemically infected Kruger National Park (KNP) and the FMD protection areas (Bruckner et al., 2002).

The beginning of the 21st Century saw numbers of FMD outbreaks in cattle within the protection zone increasing by more than one outbreak a year (Baipoledi et al., 2004; Jori et al., 2009; Thomson et al., 2013). In 2011 a SAT1 FMD outbreak was reported in the FMD free zone of Kwazulu Natal Province that led to a three-year loss of South Africa's FMD free status without vaccination causing a direct economic cost of approximately four billion Rands (DAFF, 2011). In 2014 (the year of re-instating South Africa's FMD free

[1] (https://www.nda.agric.za/vetweb/legislation/Gov%20Gaz%20-%20Act%2035%20of%201984%20-%20Part%201.pdf)

status by the OIE), the national Directorate of Animal Health issued a Veterinary Procedural Notice (VPN) for Foot-and-Mouth Disease Control in South Africa (DAFF, 2014). This VPN defined FMD control measures in the country, with an ultimate goal of protecting the FMD free status and containing the disease within the endemic areas. Disease control fences, vaccination, surveillance and movement control were the major control measures discussed in the VPN. Other control matters discussed included cattle identification and stray African buffalo control.

6.2. Veterinary fences

The FMD protection zone with vaccination (PZV) is adjacent to the western fence of Kruger National Park (KNP) and adjoining nature reserves. These private nature reserves are not considered endemic for FMD with some exception stipulated in the VPN. Game reserves adjacent to KNP (without fencing but contained in the perimeter fence of KNP) are considered FMD infected, while reserves that are not adjacent to KNP that are completely fenced and contain disease-free buffalo are not infected. Separate regulations are in place to guide breeding, and translocation of African buffaloes from the game reserves situated in the PZV to other areas of the country (DAFF, 2017). Cattle-African buffalo contact is a well-established cause of FMD occurrence in southern Africa (Vosloo et al., 2002; Thomson et al., 2003; Thomson, 2008; Jori and Etter, 2016). Thus, the contribution of cattle density to FMD occurrence might be associated with cattle incursions into KNP and wildlife leaving the reserves causing contact and possible FMD transmission (Jori et al., 2011). According to the FMD VPN and as stipulated in the Animal Disease Act (Section 18, 35/1984), disease control fences must be inspected regularly by veterinary officials.

Physical separation of wildlife and livestock is one of the most important factors of FMD control (Dion, 2012). Although fences were erected to mitigate the contact risk, the efficiency of disease control fencing in the protection zone is questionable due to several factors including increased elephant populations, increased human settlements near the fence and major flooding events (Jori et al., 2009; Scoones et al., 2010). Fences are difficult to maintain and are frequently damaged by animal, human and floods allowing cattle-African buffalo contact (Kaszta et al., 2018), which is estimated at 30-120 contacts occurring annually and about 650 African buffaloes escaping KNP a year (Jori et al., 2009; van Schalkwyk et al., 2016).

Dip-tanks that experienced FMD outbreaks were closer to KNP or private game reserve fences. Therefore, a cause of outbreaks was likely wildlife/cattle contacts due to fence permeability increasing the risk of FMD occurrence (Chapter III & V). The veterinary fence control was not covered in the VPN. The VPN mentions the role played by fences in maintaining minimal contact between livestock and wildlife (DAFF, 2014). The clause reads *"veterinary fence control, including that of animal disease control fences near the international boundary fences, the Kruger National Park veterinary fence, and fences of land registered for the keeping of African buffalo in the controlled areas, should be performed to prevent the spread of FMD from infected African buffalo populations, or from neighbouring countries, to the RSA, or the rest of the RSA, including erection, patrol and maintenance"*. However, the VPN does not provide any details on implementing the above-mentioned clause. According to the VPN, private African buffalo game reserve owners are required to erect fences and maintain their effectiveness. The legal requirements for fence erection and maintenance are included in different acts, including

the Animal Diseases Act 35 of 1984, National Environmental management: Biodiversity (NEMBA) Act 10 of 2004 and Provincial/local government legislatures (www.globalsecurity.org/military/rsa/fence-veterinary.htm).

6.3. Vaccination

Vaccination protects communal livestock from acquiring FMDV infection by reducing the number of susceptible animals in a population. Although vaccination is the second line of defense in South Africa after fencing, it is regarded as one of the most important approaches for FMD control globally (Kitching, 2005). As stipulated in the VPN, cattle in the protection zone with vaccination must be vaccinated every four months including a second primary dose 3-4 weeks later for first-time vaccinated animals. However, the effectiveness of vaccination is complicated by the antigenic variability of SAT FMDV and the uncertainty surrounding protection by currently used vaccines. Fifty-eight percent (14/24) of the evaluated SAT1 viruses screened as part of these studies had an adequate vaccine match while, only 12% (3/25) of the SAT2 isolates were antigenically similar to the vaccine strain based on the novel r_1-value calculation method (Chapter IV). This difference is likely a reflection of the high variability of SAT2 viruses (Haydon et al., 2001; Maree et al., 2011; Brito et al., 2014; Lazarus et al., 2018). Therefore, vaccine matching is an essential step to monitor the effectiveness of FMD vaccination as a control measure (Chapter IV). The availability of the vaccine and vaccination coverage varied between the two study provinces (Chapter V). Furthermore, vaccine matching results questioned the ability of the currently available vaccine to protect against circulating field viruses (Chapter IV). Although vaccine matching results were in-adequate for SAT2 circulating viruses, the

low vaccine coverage and long vaccination intervals in some areas are considered a greater risk for FMD occurrence and spread compared to the low r_1-values.

6.4. Surveillance

The VPN describes clinical, serological and virological surveillance within the PZN. Clinical surveillance is clearly defined as a routine practice (every seven days); however, the other two measures were left to be determined by the National Directorate of Animal Health on an ad hoc basis. Cattle are required to be inspected in the PZV every seven days and at least 10 randomly selected cattle are routinely mouthed on each inspection day at every inspection point (DAFF, 2014).

Although, the VPN does not include an outbreak contingency plan nor standard operating procedures for outbreak control, guidelines are provided for ad hoc development of these documents by the national and provincial authorities. Limited details are also included concerning passive and active surveillance during outbreaks. However, it is evident that serological surveillance is applied during outbreak events (DAFF, 2011).

The spatial analysis of FMD PZV outbreaks (2005 - 2016; Chapter III), revealed an apparent spatial heterogeneity in the serotype-specific risk for FMD outbreaks. Four areas at high-risk for FMD outbreaks were identified suggesting the need to develop a semi-quantitative spatial risk model. The overall predicted risk of FMD occurrence did not differ considerably from spread. However, this finding was influenced by expert opinions on the ranking of risk factors for occurrence and spread. Furthermore, variation between the two studied provinces was likely due to differences in the availability of data and the efficiency of data management between provinces (Chapter V). Outbreaks that occurred during the study period (Chapter III & V) were used to visually validate spatial risk maps. Most

outbreaks occurred in areas with relatively low predicted risk for FMD occurrence and spread, which coincided with the areas that had the highest clinical surveillance effectiveness. This finding points towards surveillance effectiveness (cattle inspection proportion) being an influential factor for FMD detection and subsequent reporting. Introducing new measures to enhance clinical surveillance effectiveness and routine laboratory-based surveillance (Teifke et al., 2012) might identify more outbreaks by recognizing currently undetected clinical cases. Outside the communal areas, serological surveillance is performed at the owner's cost, except for the first 15 samples (financial year/per owner). Serological surveillance within the communal setting is not clearly specified in the VPN and left to be determined by the national veterinary authorities upon suspicion of the disease (DAFF, 2014). However, surveillance will be performed at no cost to the communal farmer. Structured, subsidized laboratory testing might identify more FMD occurrences.

6.5. Movement control

Expert's opinions ranked *"movement into*" a village(s) as a moderate risk factor for FMD occurrence and spread, while *"movement out"* the lowest risk factor for both investigated outcomes. FMD outbreaks descriptively appeared not to be significantly different between areas with high *"movement out"* and *"movement in"* to village(s). High animal *"movement into"* a village(s) was observed in some low-risk areas for FMD occurrence and spread that actually reported outbreaks during the study period. This finding suggests the possibility that animals are moved to these areas from locations with ineffective surveillance thus increasing the risk of outbreaks.

The movement of animals is covered in detail within the VPN. The guidelines regarding live animal movement conditions are addressed in regulations 21 (1) (a). These guidelines describe when an animal can be moved and what requirements must be in place including: vaccination history of the animals, acquiring movement or red cross permits, adhering to quarantine measures, performing serological testing and having an animal identification system. Most of these measures also apply to the movement of African buffalo. No animals are permitted to exit the FMD PZV to the protection zone without vaccination or the free zone.

6.6. Recommendations

In January 2019, South Africa lost its free status without vaccination due to a SAT2 outbreak in the formally free zone of Limpopo Province (DAFF, 2019). This alarming situation requires different thinking and approaches that should lead to an improved FMD control strategy. The results of the present study suggest that the following should be performed

- Increasing government investments in veterinary services and improving the capacities of provincial veterinary services.
- Intensifying extension programmes and creating more awareness among community farmers on effective FMD control measures. Communal farmers need to be educated about the disease and its effect on the local and national economy.
- Providing training to state veterinarian and animal health technicians on data entry, storage and basic epidemiological analysis.
- Conducting periodic vaccine matching analysis to assure the effectiveness of the employed vaccine.

- Performing regular audits for the control and surveillance measures applied to ensure similar practices and implementation of FMD control measures between provinces. This measure could be applied in conjunction with the standardisation of reporting systems across all provinces using a national centralised database.
- Advancing passive and active surveillance for enhanced FMD detection capabilities including evaluating the vaccination programme (post-vaccination monitoring). Passive surveillance could encourage private veterinary clinicians to send samples to the national reference laboratory on a quarterly basis.
- Complementing clinical surveillance with periodic (every 3 monthly) serological testing using a non-structural protein ELISA, which will aid in detecting sub-clinical infections.
- Testing imported and locally manufactured vaccines according to international (OIE and FAO) standards and applying post-vaccination monitoring protocols and guidelines.
- Improving and enforcing animal identification procedures across the FMD control zones. The Livestock Identification and Traceability System (LITS) for South Africa was launched in January 2020.
- Renovating and maintaining traditional dip-tanks for effective vaccination and surveillance practices.
- Introducing risk-based surveillance to minimize cost and improve efficiency.
- Performing periodic (annual) FMD risk assessments to account for the evolving environmental, cultural and epidemiological dynamics of the disease.

- Providing farmers with compensation as incentive to maintain efficient control measures, which might lead to more effective movement control.
- Introducing mobile slaughtering facilities in the PZV to incentivize farmers affected by current FMD control measures.
- Promoting commodity-based trade to mitigate the risk of introducing FMDV to the FMD free area and subsequently contribute positively to the micro and macro economy.
- Consolidating and defining the responsibilities and roles of agencies for monitoring and maintaining the veterinary disease control fence.

6.7. Future research

This study provides preliminary findings that require further research in the following areas:

- Non-structural protein (NSP) serological surveys to assess surveillance bias to determine differences in regions with outbreaks to areas without reported outbreaks.
- Developing quantitative and predictive models of FMD risk in South Africa such as conditional autoregressive models. These models could incorporate FMD outbreak data reported between 2017 - 2020, which are more than 42 outbreaks (20 outbreaks in the free zone, while the remaining 22 were reported in the protection zone).
- Re-evaluating the FMD risk assessment model by incorporating real data on African buffalo/wildlife contacts, fence permeability and maintenance, veterinary personal demographics and serological surveillance data. These data could be collected through collaboration with the wildlife research station in the FMD endemic zone (Skukuza) and South African National Parks (SANParks) local office in KNP. Provincial veterinary services could assist with data on personnel performance and annual evaluation forms.
- Furthermore, accessing a wider pool of FMD control experts to have their opinion on ranking the risk factors for FMD occurrence and spread. The pool could be formed to include academic researchers, veterinary officials implementing the FMD control measures and international experts with knowledge concerning the epidemiology of FMD in southern Africa.

- Quantifying the effect of other disease outbreaks in the FMD control zone on routine FMD surveillance. Data could be collected by interviewing veterinarians, animal health technicians and animal assistants on their daily routine during the week compared to the designated FMD clinical surveillance day for each specific dip-tank.
- Performing a validation of the novel vaccine matching method using known vaccine seed viruses. This could be done through forming a collaborative network for data sharing with other regional, continental and global reference laboratories.
- Conducting further studies to assess the risk of FMDV introduction from Mozambique and Zimbabwe into South Africa.

6.8. Conclusion

The presented results contribute towards answering the question “how can we critically evaluate the current FMD control measures and use its outcomes to regain and maintain free status without vaccination?”. The evolving nature of the disease and its complicated epidemiology in southern Africa due to the presence of the African buffalo and the genetic variability of SAT2 viruses requires continuous research. Our study has identified some risk factors including vaccine matching, cattle inspection and movement control as drivers for FMD occurrence, detection and spread. These findings are preliminary and they require further investigation.

Successful FMD control in South Africa will have a widely beneficial outcome. Consumers will benefit from greater stability and availability of livestock products. Livestock owners will have fewer losses and greater market opportunities and the people working and running businesses in the livestock sector will have a more reliable source of products.

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Appendixes

Appendix A

Appendix A-1: Sample of Mpumalanga Province Veterinary Services report on FMD outbreak (2012)

MPUMALANGA VETERINARY SERVICES

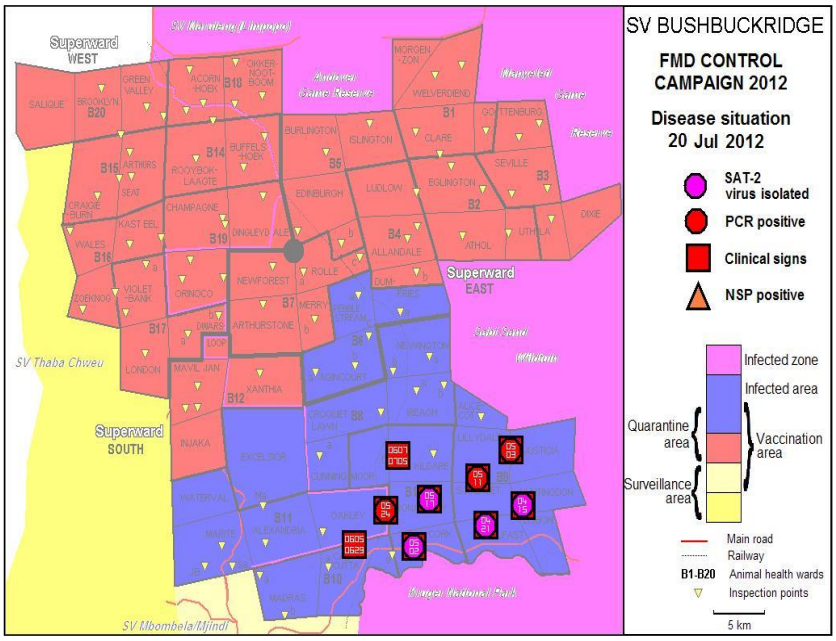
BUSHBUCKRIDGE FOOT-AND-MOUTH DISEASE OUTBREAK CONTROL CAMPAIGN 2012

FINAL INFORMATION BULLETIN NO. 13: 14 DECEMBER 2012

This is an information bulletin intended for internal use in Chief Directorates/Directorates of Veterinary Services.

Abstract

Foot-and-mouth disease (FMD) was diagnosed on 15 April 2012 in cattle at the Huntingdon communal diptank, Jongilanga traditional authority area in the B9 animal health ward in the FMD protection (formerly buffer) zone with vaccination, Bushbuckridge State Veterinarian (SV) area, Bushbuckridge local municipal area, Ehlanzeni district, Mpumalanga. On 21 April the disease was diagnosed clinically in cattle at the adjacent Belfast diptank (lesions were reported until 8 May), as well as on 2 May at the Cork diptank (lesions were reported until 30 May).



On 3 May at the Justicia diptank (lesions were reported until 10 May), on 9 May at the Somerset diptank, on 17 May at the Ronaldsey diptank (lesions were reported until 31 May), at 24 May at the Oakley B crushpen, on 5 at the Calcutta C diptank (lesions were reported until 26 June), and on 7 June at the Cunning Moor B diptank (lesions were reported until 5 July 2012).

Background

The Bushbuckridge communal grazing area is adjacent to the greater Kruger National Park and the Andover Game Reserve on its southern, eastern and northern boundary. A veterinary fence was erected along the southern boundary of the Bushbuckridge area in the early 1960's, upgraded and electrified in 1998, flood damaged in 2000, repaired in 2001, albeit without functional electrification and still permeable at some crossings of tributaries of the Sabie River, which runs in an easterly direction along and immediately south of the veterinary fence.

At the end of March 2012 there were 79 517 cattle in the area according to the official cattle registers, which are updated weekly during compulsory foot-and-mouth disease inspections. There were also 12 655 goats, 102 sheep and 3 413 pigs in the area.

Cattle in this zone are to be vaccinated against FMD three times annually, each campaign starting from January, May and September. From 1979 to 2009 vaccinations were done twice per year. Since October 2006 “Aftovax”, a trivalent vaccine imported from Botswana Vaccine Institute (BVI), has been used, after Onderstepoort Veterinary Institute (OVI) vaccine became unobtainable. Cattle are to be officially inspected weekly throughout the year in officially erected and maintained handling facilities at inspection points during fully subsidised cattle dipping. Movement control measures are in place according to the Veterinary Procedural Notice for FMD control in South Africa June 2012.

The last FMD outbreak in the Bushbuckridge SV area was detected on 1 February 2001 at the Orinoco diptank (SAT-2), after which the disease spread to 28 other diptank areas and farms. The ensuing control campaign was terminated on 31 August 2001. Earlier outbreaks occurred in 1938, 1939, 1944, 1945, 1951, 1954, 1955, 1958, 1959, 1960, 1974, 1977 and 1979.

The last FMD outbreak in the vicinity of the Bushbuckridge SV area was the unrelated SAT-2 outbreak at Spelenyane and Luphisi in the Nsikazi area on 29 December 2011, of which the control campaign is still in place.

Disease events

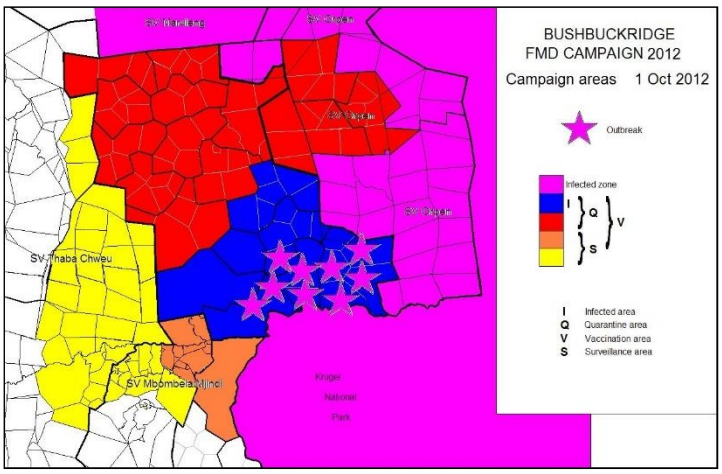
No new disease events happened. The preliminary clinical endpoint of the epidemic remained 5 July 2012.

Follow-up of suspicions

No new suspicions were detected.

Campaign areas

The campaign areas of the Bushbuckridge campaign remained as determined on 18 April 2012 (the description of the quarantine area changed due to re-aligning of SV areas on 1 October 2012) considering known disease situation, animal distribution and movements and natural and artificial barriers, until the termination of the campaign on 12 December 2012, as follows:



Infected area:

the animal health wards of *B6, B8, B9, B10, B11 and B13* of the Bushbuckridge SV area

Quarantine area:

infected area;

the remaining ten animal health wards of the Bushbuckridge SV area;

the animal health wards B1, B2, B3 and B4 of the Orpen SV area (transferred from the Bushbuckridge SV area as from 1 October 2012)

Vaccination area:

quarantine area;

the Sabie River animal health ward of the Mbombela/Mjindi (formerly Nelspruit) SV area;the farms *Sandford, The Red Ridge, De Rust* and *Perry's Farm* in the White River animal health ward of the Mbombela/Mjindi SV area

Surveillance area:

the Sabie River animal health ward of the Mbombela/Mjindi (formerly Nelspruit) SV area;

the part of the White River animal health ward of the Mbombela/Mjindi (formerly Nelspruit) SV area north of and including the farms *Burgershall, Nola, Lange Spruit* and *Duminy*;

the part of the Sabie animal health ward of the Thaba Chweu (formerly Lydenburg) SV area which is in the FMD protection zone without vaccination;

the part of the Pilgrim's Rest animal health ward of the Thaba Chweu (formerly Lydenburg) SV area which is in the FMD protection zone without vaccination.

Movement control

No veterinary roadblocks or veterinary vehicle patrols could be instituted due to lack of staff.

The only movement control that could be instituted was that no permits were being issued for livestock and product movements regarded as being of high risk and that cattle owners were requested to refrain from effecting such movements.

No movements of livestock or products were allowed within or out of the infected area or out of the quarantine area.

Since 5 September 2012 movements of live cattle for any purpose within the rest of the quarantine area have been allowed on permit on condition that the inspection and vaccination history of the herd was satisfactory.

The Selati railway line runs through the infected and quarantine areas but did not pose any significant risk.

Since the termination of the campaign on 12 December 2012, routine movement control measures according to the Veterinary Procedural Notice for Foot-and-mouth disease control in South Africa, June 2012, were in effect.

Forward tracing

No illegal movements were detected or investigated.

Surveillance

A mouthing exercise to assess whether there was still any disease present in the infected area and whether the rest of the quarantine area is still free from disease, commenced on 5 October 2012. A mouthing coverage of 38% was achieved in the infected area and 33% in the rest of quarantine area. A target of 30% coverage was set considering staff and vehicle shortages.

In the infected area routine cattle inspection and mouth and feet examination of suspect cattle continued as planned at all the inspection points on normal inspection days.

During surveillance performed in the infected area by veterinary officials from the Bushbuckridge and Orpen SV offices, no additional diseased cattle were detected up to Wednesday 12 December 2012, indicating that the former gradual westward and northward spread of the infection had been halted and that the disease seemed to have been contained.

In the rest of the quarantine area routine cattle inspection and mouth and feet examination of suspect cattle continued as planned at all the inspection points on normal inspection days.

In the surveillance area routine inspection was continued as planned.

Since the termination of the campaign on 12 December 2012, routine surveillance according to the Veterinary Procedural Notice for Foot-and-mouth disease control in South Africa, June 2012, were in effect.

Cattle and small stock surveillance statistics for the infected area, the rest of the quarantine area and the surveillance area are summarised in the table below.

Vaccination

Infected area

The first round of vaccination of cattle was done from 19 April to 1 June 2012 (7 weeks) and a vaccination coverage of 93% was achieved, the second round from 4 June to 27 July 2012 (8 weeks) reached a coverage of 95%, and the third round from 30 July to 25 October 2012 a coverage of 79% was achieved.

During the fourth and final vaccination round in cattle, which was done from 26 November 2012 a vaccination coverage of 60% was achieved.

Rest of quarantine area

Due to vaccine shortage, the first round of cattle vaccination could only commence on 4 June 2012. It lasted 8 weeks until 27 July 2012 and a vaccination coverage of 90% was achieved.

The second round of cattle vaccination was done from 30 July to 24 October 2012. A vaccination coverage of only 24% had been achieved, being low partially due to the anthrax threat from the northeast, in view of which anthrax vaccination was prioritised.

The third and final vaccination round in cattle commenced on 6 November 2012 and 71% of cattle were vaccinated.

Cattle vaccination was being done at a 5 ml (high) dose.

Vaccination statistics for the infected area, the rest of the quarantine area and the surveillance area (only applicable to the FMD protection zone with vaccination) are summarised in the table below.

Communication

No media releases were made. All queries from media were to be referred to the Director.

Twenty-two veterinary operational committee meetings were held.

Administration

No veterinary officials were detached to the campaign and only local officials from the Bushbushridge and Orpen SV offices were involved.

All material and equipment in use in the campaign were obtained from current and contingency stocks in the district and neighbouring Ehlanzeni South district.

The Bushbuckridge SV office in Thulamahashe was the campaign management centre.

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Campaign reports and SV monthly reports were used in compilation of these tables. Months end on last Friday.

INFECTED AREA: Cattle surveillance and vaccination, 15 Apr to 14 Dec 2012

Month	*Herd inspec-tions*	*Total cattle*	*Cattle inspections*	*Cattle mouth exam's*	*Cattle with lesions*	*Serum samples collected*	*Tissue samples collected*	*Blood samples collected*	*Cattle vaccinated 1st round*	*Cattle vaccinated 2nd round*	*Cattle vaccinated 3rd round*	*Cattle vaccinated 4th round*
Apr 2012	1 361	27 084	37 768 (70%)	2 136 (6%)	37	1 238 (5%)	30	20	20 946 (77%)			
May 2012	7 791	27 217	81 040 (74%)	5 240 (6%)	124	92 (0%)	26	9	4 137 (15%)			
Jun 2012	10 487	27 406	103 737 (76%)	5 258 (5%)	72	5 (0%)	6	0	148 (1%)	24 661 (90%)		
Jul 2012	7 941	27 527	86 978 (79%)	2 518 (3%)	3	5 (0%)	0	0		1 247 (5%)		
Aug 2012	7 397	27 628	95 494 (69%)	451 (0%)	0	0 (0%)	0	0			19 302 (70%)	
Sep 2012	3 848	27 517	62 431 (57%)	124 (0%)	0	0 (0%)	0	0			1 139 (4%)	
Oct 2012	2 915	27 826	72 416 (65%)	10 618 (15%)	0	0 (0%)	0	0			1 468 (5%)	
Nov 2012	1 620 [a]	28 512	106 536 (75%)	25 [a] (0%)	0	0 (0%)	0	0				4 360 (15%)
Dec 2012	79 [a]	28 823	31 246 (54%)	0 [a] (0%)	0	0 (0%)	0	0				12 676 (44%)
Total	**43 339**	**28 823**	**677 646 (70%)**	**26 370 (4%)**	**236**	**1 340 (5%)**	**62**	**29**	**25 231 (93%)**	**25 908 (95%)**	**21 909 (79%)**	**17 036 (60%)**

a = not completely recorded

INFECTED AREA: Goat surveillance, 2 Apr to 28 Dec 2012

Month	*Total goats*	*Goat inspections*	*Inspection intensity*
April 2012	5 942	4 394	74%
May 2012	5 942	4 394	74%
June 2012	5 725	4 202	59%
July 2012	5 769	4 632	80%
August 2012	5 820	4 595	63%
September 2012	5 741	4 286	75%
October 2012	5 754	4 382	76%
November 2012	5 690	4 399	62%
December 2012	5 564	3 590	65%
Total	**5 564**	**38 874**	**69%**

INFECTED AREA: Sheep surveillance, 2 Apr to 28 Dec 2012

Month	*Total sheep*	*Sheep inspections*	*Inspection intensity*
April 2012	46	46	100%
May 2012	46	46	100%
June 2012	50	50	80%
July 2012	51	51	100%
August 2012	51	51	80%
September 2012	52	52	100%
October 2012	53	53	100%
November 2012	51	51	80%
December 2012	52	50	96%
Total	**52**	**450**	**92%**

INFECTED AREA: Pig surveillance, 2 Apr to 28 Dec 2012

Month	*Total pigs*	*Pig inspections*	*Inspection intensity*
April 2012	1 256	1 154	92%
May 2012	1 256	1 154	92%
June 2012	1 187	1 183	80%
July 2012	1 145	936	82%
August 2012	1 160	952	66%
September 2012	1 130	1 053	93%
October 2012	1 199	1 187	99%
November 2012	1 225	1 225	80%
December 2012	1 257	947	75%
Total	**1 257**	**9 791**	**84%**

REST OF QUARANTINE AREA: Cattle surveillance and vaccination, 15 Apr to 14 Dec 2012

Month	*Total cattle*	*Cattle inspections*	*Cattle mouth exam's*	*Cattle with lesions*	*Serum samples collected*	*Tissue samples collected*	*Blood samples collected*	*Cattle vaccinated 1st round*	*Cattle vaccinated 2nd round*	*Cattle vaccinated 3rd round*
Apr 2012	52 396	44 668 (43%)	13 (0%)	0	0	0	0	562 (1%)		
May 2012	52 352	119 034 (57%)	801 (1%)	0	0	0	0	0 (0%)		
Jun 2012	52 570	163 166 (62%)	1 380 (1%)	0	0	0	0	40 336 (77%)		
Jul 2012	52 650	141 768 (67%)	245 (0%)	0	0	0	0	6 455 (12%)		
Aug 2012	52 978	157 253 (74%)	34 (0%)	0	0	0	0		11072 (21%)	
Sep 2012	52 902	126 659 (60%)	3 (0%)	0	0	0	0		679 (1%)	
Oct 2012	51 786	125 170 (60%)	16 864 (13%)	3	3	7	3		823 (2%)	
Nov 2012	54 685	165 041 (60%)	3 [a] (0%)	0	0	0	0			31 213 (57%)
Dec 2012	54 919	66 666 (61%)	0 [a] (0%)	0	0	0	0			7 352 (13%)
Total	**54 919**	**1 109 425 (60%)**	**19 343 (2%)**	**3**	**3**	**7**	**3**	**47 353 (90%)**	**12 574 (24%)**	**38 565 (71%)**

a not recorded completely

REST OF QUARANTINE AREA: Goat surveillance, 2 Apr to 28 Dec 2012

Month	*Total goats*	*Goat inspections*	*Inspection intensity*
April 2012	16 668	8 676	52%
May 2012	16 693	8 966	54%
June 2012	16 776	11 284	67%
July 2012	16 567	12 158	73%
August 2012	16 572	9 216	56%
September 2012	16 756	10 892	65%
October 2012	16 645	8 522	51%
November 2012	16 661	7 352	35%
December 2012	17 445	8 723	50%
Total	**17 445**	**85 789**	**59%**

REST OF QUARANTINE AREA: Sheep surveillance, 2 Apr to 28 Dec 2012

Month	*Total sheep*	*Sheep inspections*	*Inspection intensity*
April 2012	67	56	84%
May 2012	67	56	84%
June 2012	90	79	70%
July 2012	105	94	90%
August 2012	110	87	63%
September 2012	110	107	97%
October 2012	110	107	97%
November 2012	133	130	78%
December 2012	91	80	88%
Total	**91**	**796**	**82%**

REST OF QUARANTINE AREA: Pig surveillance, 2 Apr to 28 Dec 2012

Month	*Total pigs*	*Pig inspections*	*Inspection intensity*
April 2012	2 852	2 114	74%
May 2012	2 811	2 031	72%
June 2012	2 743	2 222	65%
July 2012	2 841	2 556	90%
August 2012	3 054	2 459	64%
September 2012	2 969	2 754	93%
October 2012	3 202	2 419	63%
November 2012	2 882	2 094	58%
December 2012	2 862	1 887	66%
Total	**2 079**	**20 536**	**72%**

SURVEILLANCE AREA: Cattle surveillance and vaccination, 15 Apr to 14 Dec 2012

SV area and month	*Routine FMD zone*	*Total cattle*	*Cattle inspections*	*Inspection intensity*	*Cattle vaccinated*	*Vaccination coverage*
SV Thaba Chweu	Protection without vaccination	120	120	100%	-	-
SV Mbombela/Mjindi	Protection without vaccination	323	255	79%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 759	4 778	64%	0	0%
Apr 2012		**4 202**	**5 153**	**65%**	**0**	**0%**
SV Thaba Chweu	Protection without vaccination	127	253	100%	-	-
SV Mbombela/Mjindi	Protection without vaccination	318	580	91%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 758	8 583	57%	0	0%
May 2012		**4 203**	**9 416**	**59%**	**0**	**0%**
SV Thaba Chweu	Protection without vaccination	127	218	69%	-	-
SV Mbombela/Mjindi	Protection without vaccination	319	801	100%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 767	7 211	38%	2 701	72%
Jun 2012		**4 213**	**8 230**	**41%**	**2 701**	**72%**
SV Thaba Chweu	Protection without vaccination	127	239	94%	-	-
SV Mbombela/Mjindi	Protection without vaccination	319	638	100%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 768	7 086	47%	974	26%
Jul 2012		**4 214**	**7 963**	**50%**	**974**	**26%**
SV Thaba Chweu	Protection without vaccination	127	0	0%	-	-
SV Mbombela/Mjindi	Protection without vaccination	315	870	110%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 769	11 348	60%	0	0%
Aug 2012		**4 211**	**12 218**	**61%**	**0**	**0%**
SV Thaba Chweu	Protection without vaccination	129	276	107%	-	-
SV Mbombela/Mjindi	Protection without vaccination	293	866	148%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 719	10 565	71%	1 238	33%
Sep 2012		**4 141**	**11 707**	**74%**	**1 238**	**33%**
SV Thaba Chweu	Protection without vaccination	129	0	0%	-	-
SV Mbombela/Mjindi	Protection without vaccination	277	512	92%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 726	9 769	66%	-	-
Oct 2012		**4 132**	**10 281**	**65%**	**0**	**0%**
SV Thaba Chweu	Protection without vaccination	129	4	1%	-	-
SV Mbombela/Mjindi	Protection without vaccination	281	562	80%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 627	7 037	39%	-	-
Nov 2012		**4037**	**7 603**	**40%**	**0**	**0%**
SV Thaba Chweu	Protection without vaccination	129	0	0%	-	-
SV Mbombela/Mjindi	Protection without vaccination	281	281	100%	-	-
SV Mbombela/Mjindi	Protection with vaccination	3 656	2 677	37%	-	-
Dec 2012		**4 066**	**2 958**	**38%**	**0**	**0%**
Total		**4 066**	**75 529**	**52%**	**4 913**	**66%**

SURVEILLANCE AREA: Goat surveillance, 2 Apr to 28 Dec 2012

SV area and month	*Routine FMD zone*	*Total goats*	*Goat inspections*	*Inspection intensity*
SV Thaba Chweu	Protection without vaccination	35	44	126%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	898	898	100%
Apr 2012		**933**	**942**	**101%**
SV Thaba Chweu	Protection without vaccination	35	70	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	898	162	18%
May 2012		**933**	**232**	**25%**
SV Thaba Chweu	Protection without vaccination	35	70	160%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	897	161	14%
Jun 2012		**932**	**231**	**20%**
SV Thaba Chweu	Protection without vaccination	36	72	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	900	765	85%
Jul 2012		**936**	**837**	**89%**
SV Thaba Chweu	Protection without vaccination	36	72	160%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	899	764	68%
Aug 2012		**935**	**836**	**72%**
SV Thaba Chweu	Protection without vaccination	36	72	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	899	728	81%
Sep 2012		**935**	**800**	**86%**
SV Thaba Chweu	Protection without vaccination	36	72	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	899	761	85%
Oct 2012		**935**	**833**	**89%**
SV Thaba Chweu	Protection without vaccination	36	72	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	899	627	56%
Nov 2012		**935**	**699**	**60%**
SV Thaba Chweu	Protection without vaccination	36	0	100%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	899	9	1%
Dec 2012		**935**	**9**	**1%**
Total		**935**	**5 419**	**59%**

SURVEILLANCE AREA: Sheep surveillance, 2 Apr to 28 Dec 2012

SV area and month	*Routine FMD zone*	*Total sheep*	*Sheep inspections*	*Inspection intensity*
SV Thaba Chweu	Protection without vaccination	2	4	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	100%
Apr 2012		**3**	**5**	**167%**
SV Thaba Chweu	Protection without vaccination	2	4	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	100%
May 2012		**3**	**5**	**167%**
SV Thaba Chweu	Protection without vaccination	3	5	133%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	80%
Jun 2012		**3**	**5**	**100%**
SV Thaba Chweu	Protection without vaccination	3	6	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	100%
Jul 2012		**4**	**7**	**175%**
SV Thaba Chweu	Protection without vaccination	3	6	160%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	80%
Aug 2012		**4**	**7**	**140%**
SV Thaba Chweu	Protection without vaccination	3	6	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	100%
Sep 2012		**4**	**7**	**175%**
SV Thaba Chweu	Protection without vaccination	3	6	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	100%
Oct 2012		**4**	**7**	**175%**
SV Thaba Chweu	Protection without vaccination	3	6	160%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	80%
Nov 2012		**4**	**7**	**140%**
SV Thaba Chweu	Protection without vaccination	3	0	0%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1	1	100%
Dec 2012		**4**	**1**	**25%**
Total		**4**	**51**	**138%**

SURVEILLANCE AREA: Pig surveillance, 2 Apr to 28 Dec 2012

SV area and month	*Routine FMD zone*	*Total pigs*	*Pig inspections*	*Inspection intensity*
SV Thaba Chweu	Protection without vaccination	46	82	178%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 120	1 120	100%
Apr 2012		**1 166**	**1 202**	**103%**
SV Thaba Chweu	Protection without vaccination	48	96	200%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 120	456	41%
May 2012		**1 168**	**552**	**47%**
SV Thaba Chweu	Protection without vaccination	53	101	152%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 109	445	32%
Jun 2012		**1 162**	**546**	**38%**
SV Thaba Chweu	Protection without vaccination	55	108	196%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 191	1 143	96%
Jul 2012		**1 246**	**1 251**	**100%**
SV Thaba Chweu	Protection without vaccination	55	108	157%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 181	1 133	77%
Aug 2012		**1 236**	**1 241**	**80%**
SV Thaba Chweu	Protection without vaccination	60	119	198%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 194	1 142	96%
Sep 2012		**1 254**	**1 261**	**101%**
SV Thaba Chweu	Protection without vaccination	60	119	198%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 134	1 134	100%
Oct 2012		**1 194**	**1 253**	**105%**
SV Thaba Chweu	Protection without vaccination	60	119	159%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 134	629	44%
Nov 2012		**1 194**	**748**	**50%**
SV Thaba Chweu	Protection without vaccination	60	119	198%
SV Mbombela/Mjindi	Protection without vaccination	0	0	-
SV Mbombela/Mjindi	Protection with vaccination	1 134	0	0%
Dec 2012		**1 194**	**1 253**	**105%**
Total		**1 194**	**9 307**	**79%**

Appendix A-2: Sample of Limpopo Province Veterinary Services report on FMD outbreak (2010)

FINAL REPORT ON THE GRAVELOTTE FOOT AND MOUTH DISEASE OUTBREAK AUGUST 2010

LIMPOPO PROVINCE

FINAL REPORT ON THE GRAVELOTTE FOOT AND MOUTH DISEASE OUTBREAK AUGUST 2010.

1. Background

On the 12th of August 2010 a private veterinarian was called at a farm in the Gravelotte area after a farmer noticed that some animals were showing sign of discomforts on their limbs. The private veterinarian realized that the animals were showing sign of Foot and Mouth Disease. A state veterinarians was called to the farm around three two o'clock in the afternoon. A team of veterinary officials was activated to go and investigate the problem. On arrival there were 30 heifer cattle that were separated from the rest of the herd which were salivating and lame. The heifer cattle were mouthed and lesion were seen which confirmed the suspicion of Foot and Mouth Disease. Sixteen blood serum and nine epithelium tissues were collected and. the samples were taken to Onderstepoort on the 12th of August 2010. The results were made available on the 13th of August 2010 which confirmed the animals being positive for SAT 1, SAT 2 and SAT 3

The second infection was picked up on the on Friday the 27th August 2010 the farm Sebakwe showed reactions on serology and upon investigation it was found that a group of Brahman animals showed healed FMD lesions. This group of animals was mainly cattle that were grazing around the house camps and the rest of the animals that are far away from the house camps were clean

The two farms are in the buffer zone without vaccination. The geographical location for the farms where infection was is as follows:

Malati	30 42 00 E, 23 53 00 S	80/690 (11.59%)	30
Sebakwe	30 48 00 E, 23 54 00 S	60/410 (14.6%)	11

Two buffaloes were shot in the region in April 2010 and they were all adult animals. They might have come from the Letaba Ranch camp. There were no other activities of buffaloes roaming around the area until the outbreak occurred.
A meeting of a planning team to control the disease took place on the 15th August 2010 at Gravelotte offices of Agriculture. This facility was identified as the control center from which the disease will be combated.

Processes to control the disease
Surveillance
Movement control
Vaccination

The disease was confirmed in the buffer without vaccination and that did not have any implications relating to the status of the country as far as trading with other international countries.

The source of the infection is assumed to be from stray buffalo as the virus typing indicated that the SAT 1 virus which is linked to known SAT 1 viruses in the Pafuri camp. This was a bit complicated since the farms are far from the Pafuri camps. The relationship between the Pafuri buffaloes and the farms infected is still a mystery and that was a puzzle not completed for the Gravelotte FMD outbreak 2010

GRAVELOTTE AREA 13 AUGUST 2010 TO 10 JANUARY 2011

QUARANTINE AREA FROM 13 AUGUST 2010 TO 10 JANUARY 2011

2. Initial Disease Control and Sequence of Events

An outbreak of FMD was confirmed in cattle in the Malati dairy farm on the **13th August 2010.** Animals showing typical FMD clinical signs (salivation with blisters in the mouth and tongue) a group of 30 cattle were inspected 16 blood serum and 9 epithelium tissues were sampled.
Samples were taken to Onderstepoort Exotic Disease Division where FMD virus SAT-1 SAT- 2 and SAT- 3 was confirmed on **13th August 2010**
On further surveillance, **27th August 2010** animal at the neighbouring farm from Sebakwe was also found to be infected on serology and a team was later dispatched to the farm to check the extend of the infection. The two farms were having proper fencing which played a role in terms of the control of the disease.
FMD campaign planning team visited the affected area on **15th August 2010** and a veterinary operation committee was then established on the **16th August 2010**.
A local Joint Operation Committee was established on **18 August 2006** at Gravelotte in the Municipality offices and the Agricultural offices which served as a Veterinary Operation Centre.

Movement control:

The Gravelotte and Ba-Phalaborwa area was immediately cordon off after the Joint operation centre was established from the 18 **August 2010** quarantined.
Roadblocks were put in place after a briefing of the JOC where the role players were represented

Vaccination

There were 66251 animals in the area adjacent to the infected area and these were vaccinated within August, September and October 2010 and A follow-up vaccination was done four weeks thereafter and in both vaccination rounds a vaccination % of 85 were achieved.

Our last clinical case was on the **10th of October 2010** at Malati farm on a Kudu which then set the date to declare the area free of disease to be **10th January 2011**

Date	DIP Tanks	Total Cattle	Cattle Inspected	Cattle Mouthed	Cattle Branded	Cattle Vaccinated	Sample	SS Inspected	SS Mouthed	TOTAL PIGS	Pigs Insp	% Cattle Inspected			
AUG	60	22,495	16,015	776	11,465	15,957	1,106	936	**0**	**0**	**6**	**71%**			
SEP	192	49,661	44,843	49,661	49,661	49,661	275	3,266	**328**	**123**	**123**	**90%**			
OCT	172	65,740	56,047	23,757	67	633	0	7,028	**4,727**	**76**	**76**	**85%**			
NOV	246	78,700	72,189	9	0	0	0	9,246	**266**	**336**	**336**	**92%**			
DEC	75	58,322	52,693	0	0	0	450	1,852	**0**	**80**	**75**	**90%**			
JAN	39	14,531	13,505	0	0	0	0	0	**0**	**2,524**	**2,014**	**0**	**13**	**13**	**93%**
TOTAL	**784**	**289,449**	**255,292**	**74203**	**61193**	**66251**	**1831**	**24342**	**1,831**	**24,837**	**24,342**	**5,321**	**628**	**629**	**88%**

3. INSPECTIONS

All the cattle in the proclaimed Quarantine and Surveillance Areas were inspected on a seven day interval. Six teams were set up three doing the communal dipping tanks, two on the commercial farms and one doing the infected farms. Each team was comprised of three Animal Health Technicians responsible for the inspections. Each dipping tank would have a dipping tank committee which together with the inspection team would chart the way forward as to the activities of the dipping tank on the day. On the commercial farms the teams arranges with the farmer concerned for the time of inspections.

Two supervisors were identified one for commercial and one for communal farmers; two state veterinarians were also responsible for the same job. Due to the fact that infection was only at two farms there was a team that was solely responsible for doing the two infected farms and the other team doing the clean farms in the area (commercial farms)

At a dipping tank each cattle owner will bring along a stock card for every inspections this is so in order to get the percentages of animals present on the day versus the cattle which will be absent. The inspections teams would make follow ups on cattle which are absent on the same day, next day, or during the coming week, or lastly at the next inspection dated. The percentage of animals inspected was 88%

4. VACCINATIONS

The area was vaccinated twice using the Botswana trivalent vaccine. All animals above the age of three months were vaccinated and branded with an F-brand on the right hand side of the neck as per the Animal Disease Act. We also use paint in order to identify those animals which were present and vaccinated on the day.

1st Vaccination

AREA	TOTAL	VACCINATED	BRANDED
QUARANTINE	16015	15957	11465

2nd Vaccinations

AREA	TOTAL	VACCINATED	BRANDED
QUARANTINE	49661	49661	49661

5. MOVEMENT CONTROL

The Dairy farm had a production factory that was responsible for the supply of processed products within the area and the products went through the process of pasteurization which was set at higher temperatures and longer time period as prescribed in the FMD protocol. All inter ward transfer of livestock, their products and other possibly contaminated products were prevented from leaving the area. The Gravelotte Agricultural office was used as a command center, all movement was handled by one officer. At the beginning of the campaign there were quite a lot of confiscated materials or products which was mainly due to lack of information from the community side, but with time very few products were confiscated.

Confiscated Material

Live animals	Meat(kg)	Milk(Lt)	Hides/skin	Grass	Manure (kg)
	193	62	0	1 ton plus 30 bundles	0

All materials or product confiscated were incinerated at Ba-Phalaborwa Municipality burning site on Mondays and Thursdays. The Local Municipality provided the resource for the burning of confisticated products.

6. LAW ENFORCEMENT

The community was very supportive in helping to combat the spread of the disease. We had minimal illegal movement of animals or their product within or outside the area. The commercial farmers were also helpful by using their farming organisation to alert the farmers on the measures to be taken when dealing with movement of products and live game. One challenge was the poaching of warthogs at the infected farm but to our surprise the disease still remained within the farm.

7. ROADBLOCKS

When we started with the campaign 9 roadblocks were set up around the area in order to prevent the spread of the disease to the neighbouring villages or dipping tanks and commercial farms. All roadblocks were manned for 24 hours seven days a week. Two shifts were being used for the officials manning the roadblocks. Apart from Veterinary services other role players were as follows. After 100% mouthing was conducted and no cases found our roadblocks were then reduced to 6 which were cordoning the Gravelotte and the Ba-Phalaborwa areas.

South Africa Police Services assisted in searching and providing safety at the roadblocks
Traffic Department assisted in stopping of the vehicles.
The Disaster Management Team assisted in pitching up and removal of tents.

ROADBLOCK SETTING UP

8. EXTENSION AND COMMUNICATION

We had a one official who was responsible for communicating with our community. Meetings were held with the Traditional leaders, Local Councilors, Farmers. Meetings would coincide with the imbizos of that particular village of Tribal council and a platform would be given to our offices to have a say on FMD. The mileage covered in terms of informing the community about the situation relating to the disease was very good. The whole Ba-Phalaborwa and the Gravelotte areas were addressed.

9. FINANCE AND EXPENDITURE

Total expenditure from the beginning of the campaign up to the end of it is as follows
August 2010 up to January 2011(Agriculture)

EXPENDITURE

	August-10	September-10	October-10	November-10	December-10	January-11	Accum
TRANSPORT	R 124,110.66	R 197,475.15	R 199,582.49				R 521,168.30
OVERTIME	R 797,875.51	R 1,475,594.94	R 1,307,062.55	R 1,202,132.45	R 1,098,173.14	R 417,776.66	R 6,298,615.24
S&T	R 184,758.00	R 437,741.85	R 387,302.50	R 324,725.50	R 283,369.50	R 96,436.80	R 1,714,334.15
PROCUREMENT		R 25,378.41	R 106,681.96	R 94,850.08	R 21,757.89	R 17,235.99	R 265,904.33
ACCOMMODATION	R 230,815.00	R 532,650.00	R 476,705.00	R 391,950.00	R 204,624.00	R 66,612.00	R 1,903,356.00
	R 1,337,559.17	R 2,668,840.35	R 2,000,629.49	R 1,621,708.03	R 1,403,300.53	R 531,449.45	R 9,563,487.02

10. PERSONNEL

All veterinary personnel were coming from the Limpopo Province. We were fortunate that the disease did not spread further hence there was no need to request assistance from other provinces. We had briefing sessions to update the teams about the state of the disease in the field and also team building sessions to keep them motivated.

The breakdown on personnel strength during peak activities was as follows

Veterinary : 166
SAPS : 121
Traffic : 78
Disaster Management : 06

TOTAL : **371**

11. CLOSURE.

- The area was declared free of infection on the 10th January 2011
- All control measures as prescribe by act (Act 35 of 1984) and its Regulations will be applicable on all cloven hoofed animals and their products as this area is part of a controlled zone for FMD control

DR MC MABASO
CAMPAIGN COORDINATOR
JOC CHAIRMAN

Appendix A-3: Mpumalanga Provincial Veterinary Services reporting system (sample).

WARD P1 MONTH FEBRUARY 2016 OFFICER S.D. RIKHOTSO

DIPPING OF CATTLE FOR THE MONTH
SUMMARY DIP TANK REGISTER

DIPTANK	TYPE OF DIPWASH	ADDED EACH WEEK: WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	TOTAL USED	No. DIP DAYS	PREVIOUS No. DIP DAYS	CURRENT TOTAL DIP DAYS
LULEKANI A	TAKTIC	FMD		VACCINATION					8	8
LULEKANI B	TAKTIC				3.5KG	—	3.5KG	1	7	8
BEMFARM	DELETE	FMD		VACCINATION					4	4
TOTAL					3.5KG		3.5K	1	19	20

SUMMARY OF STORES OF DIPWASH

TYPE OF DIP WASH	BALANCE BROUGHT FORWARD	RECEIVED DURING MONTH	USED DURING MONTH	BALANCE ON HAND
TAKTIC 3.5KG	7KG	—	3.5KG	3.5 KG
TAKTIC 3KG	5.5KG	—	—	5.5KG
DELETE	6960ML	—	—	6960ML

WARD P1 MONTH FEBRUARY 2016 OFFICER S.D. RUKHOTSO

DIPPING OF CATTLE FOR THE MONTH
SUMMARY DIP TANK REGISTER

DIPTANK	TYPE OF DIPWASH	ADDED EACH WEEK					TOTAL USED	No. DIP DAYS	PREVIOUS No. DIP DAYS	CURRENT TOTAL DIP DAYS
		WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5				
Lulekani A	TAKTIC	FMD	VACCINATION						8	8
Lulekani B	TAKTIC				3.5KG	—	3.5KG	1	7	8
BEMFARM	DELETE	FMD	VACCINATION						4	4
TOTAL					3.5KG		3.5K	1	19	20

SUMMARY OF STORES OF DIPWASH

TYPE OF DIP WASH	BALANCE BROUGHT FORWARD	RECEIVED DURING MONTH	USED DURING MONTH	BALANCE ON HAND
TAKTIC 3.5KG	7KG	—	3.5KG	3.5 KG
TAKTIC 3KG	5.5KG	—	—	5.5KG
DELETE	6960ML	—	—	6960ML

DIRECTORATE OF ANIMAL HEALTH : MONTHLY REPORT OF LIST A, B & C DISEASE OUTBREAKS

MONTH : (YYMM) 1602 PROVINCIAL CODE : 03 STATE VET. AREA CODE : 314

FARM ID	DISTRICT CODE	DISEASE CODE	SPECIES CODE	GEOGRAPHICAL LOCATION		NO. OF OUTBREAKS	NO. OF CASES	NO. DEAD	NO. KILLED/ SLAUGHTERED	NO. TREATED	NO. VACCINATED
				EAST	SOUTH						
LU 18	334	A01	010	3102166	2351166						
LU 18	334	A01	010	3102166	2351166						655
LU 26	334	A01	010	3102073	2353233						627
LU 18	334	A01	010	3101176	2350366						466
LU 19	334	A01	010	3057157	2350433						284
LT 746	334	A01	010	3057133	2349300						102
LT 746	334	A01	010	3057133	2349300						320
LT 745	334	A01	010	3058433	2345327						44
LT 745	334	A01	010	3058404	2347331						282
KU 9	334	A01	010	3107000	2404000						57
KU 33	334	A01	010	3104000	2400000						159
LU 30	334	A01	010	3105491	2357266						7
											799

STATE VETERINARIAN _____ DATE _____

PROVINCIAL DIRECTOR OF VETERINARY SERVICES _____ DATE _____

WARD. P1 MONTH. FEBRUARY OFFICER. S.D. RUKHOTSO

SUMMARY SMALL STOCK REGISTER.

GOATS

DIPTANK	PREVIOUS TOTAL	KIDS BORN	PERMIT IN	PERMIT OUT	DIED	SLAUGHTERED	CURRENT TOTAL	NUMBER OF OWNERS
Lulekani A.	344 ✓	NO INSPECTION DUE TO FMD					344	35
BEMFARM	88 ✓	VACCINATION					88	7
Lulekani B.	76 ✓						76	7
TOTAL	508 ✓	—	—	—	—	—	508 ✓	49 ✓
PREVIOUS TOTAL		—	—	—	—	—		
CURRENT TOTAL		—	—	—	—	—		

SHEEP

TOTAL								
PREVIOUS TOTAL								
CURRENT TOTAL								

WARD. P1

MONTH. FEBRUARY 2016

SUMMARY CATTLE REGISTER.

OFFICER. S. D. RIKITOTO

DIPTANK	PREVIOUS TOTAL CATTLE	CALVES BORN	TOTAL CALVES	PERMIT IN	PERMIT OUT	SOLD AUCTION	SLAUGHTER	DIED CALVES	DIED LARGE ANIMALS	CURRENT TOTAL CATTLE	NUMBER OF OWNERS
LULEKAMI A.	675 ✓	32	665	11	10		36	2	3	667 ✓	81 ✓
BENFARM	548 ✓	27	330	6	13		16	2	3	547 ✓	47 ✓
LULEKAMI B.	687 ✓	22	339		6		20	3	7	673 ✓	63 ✓
TOTAL	1910 ✓	81 ✓	1334	17 ✓	29 ✓	—	72 ✓	7 ✓	13 ✓		
PREVIOUS TOTAL		136 ✓	135	56 ✓	82 ✓	—	165 ✓	10 ✓	28 ✓	1887 ✓	191 ✓
CURRENT TOTAL		217 ✓		73 ✓	111 ✓		237 ✓	17 ✓	41 ✓		

P1 FEBRUARY 2016 S.D. RIKHOTSO

4. MONTHLY DISEASE REPORT

	Species	Inoculations	Out-breaks	Cases	Mortalities
Black Quarter					
Pulpy Kidney					
Botulism					
Tetanus					
Corynebacterium					
Pasteurellosis					
Mastitis					
Lumpy Skin disease					
3 day stiffsickness					
Heartwater	Cattle				
	Smallstock				
Other specify					

5. ANIMAL HUSBANDRY REPORT

DIP TANK	CASTRATIONS			DEHORNING
	CATTLE	SMALL STOCK	Other (specify)	
TOTAL				

COMUNAL GRAZING WARD - MONTHLY SUMMARY REPORT

WARD P1 MONTH FEBRUARY 2016

OFFICER S.D. RIKHOTSO

1. VACCINATIONS

DIP TANK	BLAN-THRAX	BRUCELLA	RABIES		NEW-CASTLE	HEART-WATER	LUMPY SKIN DISEASE	FOOT AND MOUTH DISEASE
			DOGS	CATS				
LEELSPRUIT A								284
ZEBRA								102
RHODA								159
WISELSTEEK								79
SCHELDING A								320
SCHELDING B								44
SCHALK								7
SCHIETDGHT					1			233
TOTAL	—	—	—	—	—	—	—	1228
PREVIOUS TOTAL	—	—	—	—	—	—	—	—
CURRENT TOTAL	—	—	—	—	—	—	—	—
VACCINE ON HAND								

2. DOSING FOR INTERNAL PARASITES

DIP TANK	CATTLE	GOATS	SHEEP	PIGS
N/A	N/A	N/A	N/A	N/A

3. EXTENSION SERVICES

DIP TANK	Farmer's days	Attendance	Extension meetings	Attendance
N/A	N/A	N/A	N/A	N/A

8

WARD B10

MONTH:DECEMBER

DIPTANK/ FARM/ PLACE	ZONE (INF, BV, BN, FREE)	GOATS			SHEEP			PIGS			BUFFALO			OTHER				OTHER INSPECTIONS	DIPTANK MAINTENANCE			
		Inspected	Total goats (end of month)	Total goat owners	Inspected	Total sheep (end of month)	Total sheep owners	Inspected	Total pigs (end of month)	Total pig owners	Inspected	Total buffalo	Total owners	Species	Inspected	Total	Total Owners		Dip wash analysis	Tick Samples Collected	Loose Poles Repaired	Firebreak Cleared	Comments on Diptank Maintenance
MADRAS A		0	72	21	0	38	4																
CALCUTTA A		0	87	18	0	73	6																
CALCUTTA E		0	0	6	0	102	2																
OAKLEY B		0	87	16	0	49	11																
MADRAS B		0	34	6	0	49	2																
MONTH TOTAL		0	352	67	0	311	25																
QUARTER TOTAL		###																					
YEAR: PREVIOUS TOT		###	475		965	683																	
YEAR: NEW TOTAL																							
YEAR OBJECTIVE																							

7	WARD B9	MONTH:OCTOBER	CATTLE DIPPING & INSPECTION

B	ZONE (INF, BV, BN, FREE)	WEEKLY DIP TOTALS														MONTHLY TOTALS				Potential Inspection days	MONTHLY INCREASES/DECREASES									
		WEEK 1			WEEK 2			WEEK 3			WEEK 4			WEEK 5		Total Inspections	Total Cattle Dipped	Type 1	Type 2		Total Cattle (start of month)	INCREASES			DECREASES				Total Cattle (end of month)	Total cattle owners
		Inspected	Dipped	Dipstuff Used	Inspected	Dipped	Dipstuff Used	Inspected	Dipped	Dipstuff Used	Inspected	Dipped	Dipstuff Used	Inspected	Dipped			Total Dip Used	Total Dip Used			Calf born	Permit In	From	To	Permit Out	Died	Slaughtered		
MADRAS A	BWV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0
CALCUTTA A	BWV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0
CALCUTTA E	BWV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0.0
OAKLEY B	BWV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0.0
MADRAS B	BWV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0.0
TOTALS	BWV	0	0		0	0		0	0		0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	6	0	0	0.0
QUARTER TOTAL																														
EAR:PREVIOUS TOTAL															53349	20647	34L	134L	58	16859	98	65	9	9	28	27	110	16792	1936
YEAR: NEW TOTAL															53349.0	20647.0		143L	58	16859	98	65	9	9	28	33	134	16792	1936.0
YEAR OBJECTIVE																														

DIPPING COMPOUND REGISTER

Compound Name	Type	Previous on hand	Received	Issued	Present on hand
DELETE X5	1	NIL	20L	9L	11L
DELETE	2	NIL	NIL	NIL	NIL

2b	NAME	MONTH
	BILA	DECEMBER

VACCINATION OF CONTROLLED & NOTIFIABLE DISEASES

DATE	OWNER & PLACE	REGNO.	Species	Name of vaccine	Supplier of vaccine	Number of Herds	Rabies (Dog)	Rabies (Cat)	Rabies (other)	FMD	F-branded	CA (S19)	CA (RB 51)	B.mellitensis	Anthrax	Newcastle	AHS	RVF	LSD	Blue Tongue	Eq. Influenza	Disease Name	Amount
							Controlled Diseases											Notifiable				Other	

	Rabies (Dog)	Rabies (Cat)	Rabies (other)	FMD	F-branded	CA (S19)	CA (RB 51)	B.mellitensis	Anthrax	Newcastle	AHS	RVF	LSD	Blue Tongue	Eq. Influenza	Disease Name	Amount
MONTH TOTAL																	
QUARTER TOTAL			189	5253													
YEAR: PREVIOUS TOTAL			194	5253													
YEAR: NEW TOTAL																	
YEAR: OBJECTIVE																	

Vaccine Register

TYPE	PREVIOUS ON HAND	RECEIVED	ISSUED	PRESENT ON HAND
Rabies	501	0	1	50
FMD	11409	~~48~~	~~55~~	1140
Anthrax	750	0	0	75
S19	800	0	0	80
RB 51	0	0	0	

2a	NAME:	BILA		INSPECTIONS AND LIVESTOCK AUCTIONS														DIPPINGS	
DATE	DECEMBER			CATTLE			SHEEP		GOATS		PIGS			OTHER				DIP USED	
			FARM/DPTANK/PLA	TOTAL	INSPECTION	DIPPED	TOTAL	INSPECTION	TOTAL	INSPECTION	TOTAL	INSPECTION	SPECIES	TOTAL	INSPECTED	AUCTION ATTENDED	CATTLE DIPPED	DELETE	DELETE X 5
2/12/2013	TRANSPORT PROBLEM		MADRAS A	0	0	0											0	0	0
3/12/2013	TRANSPORT PROBLEM		CALCUTTA A	0	0	0											0	0	0
4/12/2013	TRANSPORT PROBLEM		CALCUTTA E	0	0	0											0	5L	0
5/12/2013	TRANSPORT PROBLEM		OAKLEY B	0	0	0											0	0	0
6/12/2013	TRANSPORT PROBLEM		MADRAS B	0	0	0											0	0	0
9/12/2013	TRANSPORT PROBLEM		MADRAS A	0	0	0													
10/12/2013	TRANSPORT PROBLEM		CALCUTTA A	0	0	0											0	0	0
11/12/2013	TRANSPORT PROBLEM		CALCUTTA E	0	0	0											0	0	0
12/12/2013	TRANSPORT PROBLEM		OAKLEY B	0	0	0											0	0	0
13/12/2013	TRANSPORT PROBLEM		MADRAS B	0	0	0											0	0	0
																			0
16/12/2013	TRANSPORT PROBLEM		MADRAS A	0	0	0											0	0	0
17/12/2013	TRANSPORT PROBLEM		CALCUTTA A	0	0	0											0	0	0
18/12/2013	TRANSPORT PROBLEM		CALCUTTA E	0	0	0											0	0	0
19/12/2013	TRANSPORT PROBLEM		OAKLEY B	0	0	0											0	0	0
20/12/2013	TRANSPORT PROBLEM		MADRAS B	0	0	0											0	0	0
				0	0	0											0	0	0
Delete																			
X5																			

8/4/2009 10/1 MR 1

MPUMALANGA

DIRECTORATE OF VETERINARY SERVICES
MONTHLY REPORT
ANIMAL HEALTH TECHNICIAN

NAME	BILA	AHT AREA	WARD ~~B3~~ 10	MONTH	DECEMBER
RANK	CHIEF	SV AREA	BUSHBUCKRIDGE	FROM	2.12.2013
SIGNATURE		DATE	27.12.2013	TO	27.12.2013

CHECKED	CONTROL AHT	SV
COMMENTS		
SIGNATURE		
DATE	07-01-2014	

HIGHLIGHTS

NIL REPORT

DIP AND DIPPING1. DIP STORED AT OFFICE OF THE AREA

Kind of Dip	Previous Balance At office	Dip received			Distribution To dip tanks	Dip received From dip tanks	Dip sent back to vet. Main store			Present balance at office
		Quantity	Date of issued	Issue voucher No.			Quantity	Date	Issue Voucher	
Taktik				-		-		-	-	
-	-	-	-	-	-	-	-	-	-	-
Delete		-	-	-	-	-	-	-	-	

2 .DIP STORED AT THE DIPPING TANKS

Dip tank	Dip tank capacity	Present No .of cattle	Date of last visit by AHT previous Month	No. of dipping by:		Times not Dipped-Reasons	Kind of Dip used	Previous Dip balance	Dip received	Dip used	Dip balance	Date of his Last Visit	Degree of tick infestation	
				AHT	Assistant									
MASETONI		276	23/03/2015			Raining	DELETE	20			20xl	23/03/2015	Less infestation	
LAMVI	12500	870	24/03/2015				TaktiC	6x3kg		1x3kg	5x3kg	21/04/2015	Less infestation	
VHURIVHURI	17500	702	25/03/2015				TaktiC					08/04/2015	Less infestation	
MAVUNDE	12500	393	29/02/2015			No water	TAKTICS					29/02/2015	Less infestation	
MAHUNGUWI	12500	346	27/03/2015				Taktik	5X3KG			5x3kg	27/03/2015	Less infestation	
SAMBANDOU	17500	993	31/03/2015				Taktik					31/03/2015	Less infestation	
TSHANZHE	12500	399	18/03/2015				Taktik	2X3KG		2x3kg		18/03/2015	Less infestation	
TSWERA		324	05/03/2015				Delete	15XL				05/03/2015	Less infestation	
MAKONDE		172	24/07/2014				DELETE					24/07/2014	Less infestation	
MBAHELA		244	14/11/2014				DELETE					14/11/2014	Less infestation	
						Taktics	3kg	13x3kg		3x3kg	10x3kg		Taktics	10x3kg
						Taktik 3.5kg							Taktik	
						Delete		35XL			35xl		Delete	35xl

8/4/2009 10/1 MR 1

MPUMALANGA

DIRECTORATE OF VETERINARY SERVICES
MONTHLY REPORT
ANIMAL HEALTH TECHNICIAN

NAME	BILA	AHT AREA	WARD ~~B3~~ 10	MONTH	DECEMBER
RANK	CHIEF	SV AREA	BUSHBUCKRIDGE	FROM	2.12.2013
SIGNATURE		DATE	27.12.2013	TO	27.12.2013

CHECKED	CONTROL AHT	SV
COMMENTS		
SIGNATURE		
DATE	04-01-2014	

HIGHLIGHTS

NIL REPORT

Appendix A-4: Limpopo Provincial Veterinary Services reporting system (sample).

PERIOD: | MONTHLY REPORT | PRODUCTION UNIT

Date	Farm/Place	No.	Owner	INSPECTIONS Cattle	Sheep	Goats	Ekwide	Pigs	Dogs	TB Tested	CA Tested	Kilometres	Remarks
02/01/15	Malale											38	Pre-Slaughter inspection & monitoring
05/01/15	Malale/nedlene											57	Fence patrol
06/01/15	Jeanette											9	Putting on notice boards
07/01/15	Redlane											42	Fence repair
08/01/15	Musina											159	Submission of leave form
09/01/15	Nkanandi											–	Office work
12/01/15	Festeacel			275								28	Inspection & dipping [illegible] inspection
13/01/15	Masisi											95	Attend FMD meeting held there
14/01/15	Redlane											–	Completion of leave
15/01/15	Masisi											–	Attend FMD meeting
16/01/15	Masisi											–	Attend Imbizo by the Minister
19/01/15	Festeacel			170								28	Less ticks inspection
20/01/15	Antonvilla			–								212	42 cattle ear tagged
TOTALS:												668	

Jan – 2015

DESCRIPTION	PRE	PRES	ACC	DESCRIPTION	PRE	PRES	ACC
Educational :				Vaccinations :			
Demonstrations, Talks, Video/Slide shows.				Anthrax			
Exhibitions				Blantbrax			
Farmers days organized				Brucellosis			
Pamphlet distribution				Rabies : Dogs			
Communication :				: Cats			
Personnel Meetings				: Cattle			
Organized Agricultural Meetings				Black Quarter			
Meetings with other Departments/Organizations				Botulism			
Posters to advertise vaccination programs.				Black Quarter/Botulism			
Regulated Functions :				Lumpy skin disease			
Inspections : Cattle				New Castle disease : Fowls			
: Smallstock				: Ostrich			
: Pigs				Heartwater			
: Piggeries				Blue tongue			
: Game				Pulpy Kidney			
: Ostrich				Pasteurella			
Stock Sales attended : Cattle				Vitamins injected			
: Smallstock							
: Game							
Roadblocks							
Visit Vaccine Depots				Eradication schemes :			
Sampling:				TB. Tuberculin administered			
Post mortems				TB. Tests done			
Serology samples taken				TB. Positive animals branded			
Histological samples taken				TB. Positive animals slaughtered			
Smears made				CA. Blood samples taken			
Faeces samples/ egg counts				CA. Milk samples taken			
Sheath washes				CA. Positive animals branded			
Services Rendered:							
Dosing : Cattle							
: Smallstock							
: Ostriches							
Castrations : Cattle							
: Smallstock							
Dehorning							
Applying Registered Brands							
Applying Eartags							
Pregnancy Tests							
Artificial Inseminations done							
Clinical cases attended to							

ACCUMULATIVE STATISTICS:ANIMAL HEALTH WORK

Action	Current Total	Previous Total	Accumulative Total	Action	Current Total	Previous Total	Accumulative
INSPECTIONS				**ERADICATION SCHEMES**			
Cattle	4 416	4 434	8 856	TB:Herds Tested			0
Smallstock			0	TB:Herds Positive			0
Accredited Pigs			0	TB:Cattle tested			0
Other Pigs Inspected			0	TB:Cattle Negative			0
Accredited Piggeries			0	TB:Cattle Positive			0
Other Piggeries			0	TB:Cattle suspect			0
Game			0	TB:Pos.Cattle Branded			0
Ostriches			0	TB:Pos.Cattle Slaughtered			0
Buffalo Inspected			0	TB:Pos.Herds Declared Negative			0
Products Inspected			0	TB:Declaration Certificate Issued			0
Poultry Projects Inspected			0	TB:Compensation Paid			0
Poultry Inspected			0	Properties Disinfected			0
Goat Projects Inspected			0	CA:Herds Tested			0
Quarantine Inspections			0	CA;Herds Positive			0
Abattoir Permit Control Inspections			0	CA:Cattle tested			0
V.P.H. Insp dane			0	CA:Cattle Negative			0
Vet Facilities Inspected			0	CA:Cattle Positive			0
New Taxidermies Approved			0	CA:Cattle Suspect			0
New Abattoirs Approved			0	CA:Pos.Cattle Branded			0
New Meat Export Farms			0	CA:Pos.Cattle Slaughtered			0
New Diptanks/Crushpens Approved			0	CA:Pos.Herds Declared Negative			0
New Quarantine camps Approved			0	CA:Declaration Certificates Issued			0
Road Blocks Organised			0	TB/CA Campaigns			0
Quarantine Notices Issued			0				0
Official Warnings Issued			0				0
Auctions Monitored			0				0
Animals On Sales			0				0
Number of epi units visited			0				0

PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE

SV OFFICE: Nwanedi
DISTRICT: Vhembe
MUNICIPALITY: Musina
PERIOD: 26/07/15 - 25/08/15

PERMIT REGISTER

DATE OF ISSUE	ISSUING OFFICER	ISSUING OFFICE	PERMIT NO	NAME OF APPLICANT	TYPE OF PRODUCT/ANIMAL	FARM/PLACE OF ORIGIN	DISTRICT	FARM/PLACE OF DESTINATION	DISTRICT	TOTAL PRODUCT/ ANIMAL	NO. OF PERMITS	OFFICIAL'S SIGNATURE
23/07/15	Manenzhe	Nwanedi	Mus3/80	Rino project	Cattle	Suzetta	Musina	Polokwane	Capricon	23	1	
23/07/15	Manenzhe	Nwanedi	Mus3/81	Rino project	cattle	Olympie	Musina	Polokwane	Capricon	10	1	
27/07/15	Manenzhe	Nwanedi	Mus3/82	lambani	Cattle	Suzette	Musina	Ishiterete	Thulamela	4	1	
27/07/15	Manenzhe	Nwanedi	Mus3/83	Manyunga S	carcass	Festraeul	Musina	Mudimba	Musina	1	1	
29/07/15	Manenzhe	Nwanedi	Mus3/84	Nekhumbe	Sheep	Dombeni	Musina	Thohoyandou	Thulamela	1	1	
30/07/15	Manenzhe	Nwanedi	Mus3/85	Mudumela	Carcass goats	Ettie	Musina	Ishitomboni	Makhado	3	1	
03/08/15	Manenzhe	Nwanedi	Mus3/86	Mmeregi F.C	Carcass	Dombeni	Musina	Shileptene	Thulamela	1	1	
03/08/15	Manenzhe	Nwanedi	Mus3/87	Dalla S.U	carcasses	Zisaan	Musina	Thohoyandou	Thulamela	4	1	
03/08/15	Manenzhe	Nwanedi	Mus3/88	Mr Mudumela	Hides	Zisaan	Musina	Makhado	Makhado	4	1	
03/08/15	Manenzhe	Nwanedi	Mus3/89	Mr Mudumela	goat	Ettie	Musina	Maungani	Thulamela	1	1	
03/08/15	Manenzhe	Nwanedi	Mus3/90	Mr Musethwa	carcass	Hetty	Musina	Thohoyandou	Thulamela	2	1	
06/08/15	Manenzhe	Nwanedi	Mus3/91	Mphigelale	goats	Bally-farm	Musina	Vhufuli	Thulamela	2	1	
07/08/15	Manenzhe	Nwanedi	Mus3/92	Negotel G	Cattle	Irwida	Musina	Mampakuil	Thulamela	5	1	
11/08/15	Manenzhe	Nwanedi	Mus3/93	Rino Project	Cattle	Ministerring	Musina	Polokwane	Capricon	15	1	
11/08/15	Mulibana	Nwanedi	Mus3/94	Netshivumbe	Goats	Ministerring	Musina	Lwamondo	Thulamela	3	1	
12/08/15	Manenzhe	Nwanedi	Mus3/95	Dalla S.U	Carcasses	Irwida	Musina	Thohoyandou	Thulamela	3	1	
12/08/15	Manenzhe	Nwanedi	Mus3/96	Mudumela	Hides	Irwida	Musina	Makhado	Makhado	3	1	
									TOTAL	85	17	0

MASHABA RD ANIMAL PRODUCTION STATISTICS NOVEMBER 2015

DIPPING TANK	PREVIOUS CATTLE TOTAL	CALVES BORN	PERMIT IN	PERMIT OUT	SOLD AT STOCK SALES	DEAD	SLAUHTERED				TOTAL DEAD & SLAUGHTERED	TOTAL CALVES	PRESENT GRAND TOTAL	NO. OF OWNERS	NO. OF SMEARS	NO. OF NO SMEARS
						CALVES	CATTLE	TOTAL	BY OWNER	BY BUTCHER						
NTLHAVENI C	534	2							3		3		533			
BEVHULA	845	1					2	2	2		4		842			
NKAVELE	785	2							1		1		786			
MAKULEKE	1630	1							10		10		1621			
MAVILIGWE	640												640			
TOTAL	4434	6			-		-2	2	16	-	18		4422	654	-	16

Period	Nov-15		MONTHLY REPORT

		PRODUTION UNIT

INSPECTION AND WORK DONE

DATE	FARM/PLACE	NO	0WNER	CATTLE	S/STOC	PIGS	FOWLS	DOGS	CATS	TB test	CA Test	FARMERS	Remarks
2.11.15	NTLHAVENI C		109	407									F/BRANDING MEETING $ INSP
3.11.15	BEVHULA			70									MINERAL SUPPLEMENTATION
4.11.15	NKAVELE			25									LUMPY SKIN VACCINATION
5.11.15	MAKULEKE			1300									INSPECTION $ DIPPING
6.11.15	MAVILIGWE			24									VITAMIN A SUPPLIMETARY
9.11.15	NTLHAVENI C			399									INSPECTION $ DIPPING PROLAP
10.11.15	BEVHULA			29									DEWORMING,DIPPING $ INSP
11.11.15	NKAVELE			12									VITAMIN A SUPPLIMETARY
12.11.15	MAKULEKE			14									VITAMIN A SUPPLIMETARY
13.11.15	MAVILIGWE			19									DEWORMING,DIPPING $ INSP
16.11.15	NTLHAVENI C			407									INSP,DIP $BROKEN POLES CALC
17,11,15	BEVHULA			13									DEWORMING,DIPPING $ INSP
18.11.15	NKAVELE			605									INSP,DIP $BROKEN POLES CALC
19.11.15	MAKULEKE			1129									INSP,DIP $BROKEN POLES CALC
19.11.15	MAVILIGWE			312									INSP$DIPPING
	TOTAL			4765	0	0	0	0	0	0	0	0	

CA. Blood samples taken	—	—	—	
CA. Milk samples taken	—	—	—	
CA. Positive animal branded	—	—	—	
BAFFALOE T.B	—	—	—	
BAFFALOE CA	—	—	—	
SWINE FEVER	—	—	—	
FMD BLOOD CATTLE	76	—	78	
FMD BLOOD SMALL STOCK	—	—	—	
FMD BLOOD GAME	—	—	—	
PERMITS ISSUED FOR	~~150~~			
1. Cattle	150	24	174	
2. Small stock	59	35	94	
3. Game	—	—	—	

Permits Issued:

Cloven-hoofed animals need a veterinary permit to be transported or moved within, into, or out of the Foot and Mouth Disease control area.

PERMITS ISSUED	LIVE LIVESTOCK		LIVE GAME		PRODUCTS
	NO. PERMITS	NO. OF ANIMALS	NO. PERMITS	NO .OF GAME	NO. PERMITS
	48	148	NIL		1
TOTAL	48	148	—	—	1

Pig Products

	WILD PIG CARCASSES				OTHER PRODUCTS
	OUT OF PROVINCE		WITHIN PROVINCE		
MUNICIPALITY	PERMITS	CARCASS	PERMITS	CARCASS	PERMITS
MUSINA		NIL			
TOTAL					

Services Rendered :				
Dosing	: Cattle	—	—	—
stock	: Small	—	—	—
	: Ostriches	—	—	—
Castrations	: Cattle	—	—	—
stock	: small	—	—	—
Dehorning		9	—	9
Applying Registered Brands		10	—	10
Applying Ear tags		368	—	368
Pregnancy Tests		—	—	—
Artificial Inseminations done		—	—	—
wounded infections treated		—	—	—
Dystocia assisted		1	—	1
Clinical		1	—	1
VACCINATIONS :				
Foot and Mouth		—	—	—
Anthrax		—	—	—
Blanthrax		—	—	—
Brucellosis		—	—	—
Rabies	: Dogs	507	—	507
	: Cats	37	—	37
	: Cattle	—	—	—
Black Quarter		—	—	—
Botulism		—	—	—
Black Quarter/ Botulism		—	—	—
Lumpy skin disease		386	—	386
New castle		—	—	—
Heart water		—	—	—
Blue Tongue		—	—	—
Pulpy Kidney		—	—	—
Pasteurella		—	—	—
Vitamins injected		—	—	—
Eradication schemes :		—	—	—
TB. Tuberculin administered		—	—	—
TB. Tests done		—	—	—
TB. Positive animals branded		—	—	—
TB. Positive animals slaughter		—	—	—

Statistical data:

DESCRIPTION	PREVIOUS TOTAL	PRESENT TOTAL	ACCUMULATIVE TOTAL	TARG POPU
Educational :				
Demonstrations. Talks. Video /Slide shows	—	—	—	
Exhibitions	—	—	—	
Farmers days organized	—	—	—	
Pamphlet distribution	200	1	1	
Communication :		—	200	
Personnel Meetings	—	—	—	
Organized Agricultural Meetings	—	1	1	
Meetings with other Departments/ Organizations	— 2	—	2	
Posters to advertise vaccination programs	200	—	200	
Regulated Functions :				
Inspections : Cattle	8181	445	8626	
: Small stock	—	—	—	
: Game	—	—	—	
: Pigs	—	—	—	
: Approved Piggeries	—	—	—	
: Ostrich	—	—	—	
Stock Sales attended : Cattle	—	—	—	
: Small stock	—	—	—	
: Game	—	—	—	
Roadblocks	—	—	—	
Visit Vaccine Deports	—	—	—	
Sampling:	—	—	—	
Post mortems	—	—	—	
Serology samples taken	78	—	78	
Histological samples taken	—	—	—	
Smears made	—	—	—	
Faecal samples/ egg counts	—	—	—	
Sheath washes	—	—	—	
	—	—	—	

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE

MONTHLY REPORT

Name	P. Joubert					
Rank	AHT	Sv area	MUSINA			
Period	From : 2015/3/20		To :			
Summary				Days		
				Current	Accumulative	Total
Field work						
Office						
Leave						
Kind	From	To				
Vacation						
Vacation						
Vacation						
Sick-leave	2015/3/18	2015/3/18	1 Day			
Signature						
Official	P Joubert			Date	2015/3/24	
Supervisor				Date		
State VET				Date		
Remarks						

DIRECTORATE OF ANIMAL HEALTH: MONTHLY REPORT OF LIST A&B DISEASE OUTBREAKS

MONTH:(YYMM) 1503 PROVINCIAL CODE 341 STATE VET. AREA CODE 309

FARM ID	DIST CODE	DISEASE CODE	SPECIES CODE	GEOGRAPHICAL LOCATION		NO OF OUTBREAKS	NO OF CASES	NO DEAD	NO KILLED	SLAUGHTERED	NO TREATED	NO VACCINATE
				EAST	SOUTH							
MS60	341	80580	16	3006	2230							9
MS60	341	80580	17	3006	2230							2

STATE VETERINARIAN

2015/3/24
DATE

REGIONAL DIRECTOR

DATE

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE

PERIOD	MONTHLY REPORT	PRODUCTION UNIT

				INSPECTIONS									
	Date	Farm/place	No	Owner	Cattle	Sheep	Goats	Pigs	Dogs	TB Tested	CA Tested	KM	Remarks
2015	2/23	Musina		Kantoor.									Kantoor.
015	2/24	Musina		Kantoor.									Kantoor.
015	2/25	Musina		Kantoor.									Kantoor.
015	2/26	Musina		Kantoor									Kantoor.
015	2/27	Meldrake		by Minnaar.						7	7		TB LA Cowich
15	3/02	Meldrake.		by Minnaar.									TB Lees Baybo
015	3/03	Musina		Kantoor									Kantoor.
015	3/04	HAURE		Rolofse.									Vac 2 dogs
	"	"		"									Rabais.
015	3/5	Musina											Kantoor.
15	3/6	ERank		HAURE									Vac 3 dogs
	"	"		"									Rabais
15	3/9	Musina											Kantoor.
15	3/10	HAURE		Joubert									Vac 2 Cat 4
	"	"		"									Dogs Rabies
015	3/11	Musina		Kantoor.									Kantoor.
015	3/12	Concordia		Kruger.									abloard Buffalo
	"	14 days											Broke peek.
15	3/13	Musina		Kantoor									Kantoor.
15	3/16	Musina		Kantoor									Kantoor.
15	3/17	Musina		Kantoor									Kantoor.
15	3/18	Leafe.											Sick Leafe.
15	3/19	Musina		Kantoor									Kantoor.
15	3/20	Musina		Kantoor.									Does. P.I

MAANDVERSLAG
MONTHLY REPORT

NAME/NAAM: MULIBANA A D

RANG/RANK: A.H.T SV Area: MUSINA

TYDPERK/PERIOD: Vanaf/From 26/12/14 Tot/To 25/01/15

OPSOMMING/SUMMARY	Dae/Days		
	Current Huidige	Accumulative Oplopend	Total Totaal
Veldwerk/Fieldwork	12	100	112
Kantoor/Office	1	44	45

VERLOF/LEAVE					
Soort/Kind	Vanaf/From	Tot/To			

HANDTEKENING/SIGNATURE

BEAMPTE/OFFICIAL: [signature] Datum/Date: 21/01/15

TOESIGHOUER/SUPERVISOR: Datum/Date:

STAATSVEEARTS/STATE VETERINARIAN: Datum/Date:

OPMERKINGS/REMARKS

Action	Current Total	Previous Total	Accumulative Total	Action	Current Total	Previous Total	Accumulative
BUFFALO WORK & SURVEYS				**VACCINATIONS**			
Buffalo 4th and 5th stage Projects			0	Anthrax	4 419		4 419
Buffalo Released :4 & 5th Stages			0	Brucellosis	150		150
Buffalo Area Total			0	RB 51			0
Buffalo:Total Properties Registered			0	Rabies Dogs			0
Buffalo New Properties Registered			0	Rabies Cats			0
Buffalo Samples Corridor			0	Rabies Cattle & SS& Other			0
Buffalo Sammples FMD			0	Foot and Mouth Disease Cattle			0
Buffalo Samples Tuberculosis			0	Foot and Mouth Disease Smallstock			0
Buffalo Samples:CA			0	New Castle Fowls			0
Buffalo Tested			0	New Castle Ostriches			0
Buffalo Positive:CA			0	A.H.Sickness			0
Buffalo Positive:Corridor			0	Anaplasmosis			0
Buffalo Positive:FMD			0	Black Quarter			0
Buffalo Positive:Tuberculosis			0	Blue Tongue			0
Buffalo Moved			0	Botulism			0
Buffalo:Trucks Sealed:Loaded			0	BVD			0
Buffalo:Trucks Unsealed:Unloaded			0	Camphylobacter(Vibio)			0
Buffalo:Units Inspected			0	Clostridium."Dikkop'			0
Survey:African Swine Fever			0	Coryne Bacterium			0
Survey:Avian Influeza Fowls			0	Elephant Disease			0
Survey:Avian Influeza Ostriches			0	Exteroxcemia(Pulpy Kidney)			0
Survey:Classical Swine Fever			0	Enzootic Abortion			0
Survey:Foot and Mouth Disease			0	Gumborro Disease			0
Survey:Theileria Samples			0	Heartwater			0
Survey:New Castle Disease Fowls			0	Lumpy Skin	176	170	346
Survey:New Castle Disease Ostrich			0	Pasteurelosis			0
Disease Campaigns			0	Redwater			0
			0	Rift Valley Fever			0
			0	Tetanus			0
			0	Vibrio			0
			0	Vitamins			0
			0	Distemper			0

7. ANIMAL PRODUCTION STATISTICS.

Dipping tank	Provincial cattle total	Calves born	Permit In	Permit Out	Sold at stock sales	DEAD Calves	DEAD Cattle	DEAD Total	SLAUGHTERED By Owner	SLAUGHTERED By Butcher	Total dead & slaughtered by owner	Total calves	Present grand total	Number of or-	Number of	N
ESTERAAL	405	9	17	11	—	—	—	—	—	—	—	133	420	30	—	—
ETTIEMINE	654	—	—	—	—	—	—	—	—	—	—	20	654	4	—	—
CHARLOTTE	584	—	—	—	—	—	—	—	—	—	—	166	584	3	—	—
LAURA	582	—	—	—	—	—	—	—	—	—	—	103	582	4	—	—
DOMBONTI	734	—	—	—	—	—	—	—	—	—	—	24	734	37	—	—
CATOSMUTI	418	—	—	—	—	—	—	—	—	—	—	83	418	5	—	—
JEANETTE	73	—	—	—	—	—	—	—	—	—	—	10	73	1	—	—
ARMISTICE	78	—	—	—	—	—	—	—	—	—	—	—	78	1	—	—
IRRIGATION	267	—	—	—	—	—	—	—	—	—	—	62	267	5	—	—
HETTY	559	—	—	—	—	—	—	—	—	—	—	140	559	37	—	—
TAL	4354	9	17	11	—	—	—	—	—	—	—					
AND TOTAL PREVIOUS ITH						5	17	22	—	—	22	1144	4369	127	—	—
ESENT GRAND TOTAL						5	17	22	—	—	22				—	22

Appendix B

Appendix B-1: Animal Ethics Committee and Section 20 of the Animal Disease Act (Act 35 of 1984) approvals for the animal challenge study

ARC-ONDERSTEPOORT VETERINARY INSTITUTE
LNR-ONDERSTEPOORT VEEARTSENYKUNDE-INSTITUUT
Private Bag / Privaatsak X05, Onderstepoort 0110, South Africa / Suid-Afrika
Tel: (012) 529-9111 • Fax: (012) 565-6573 (Int: + 27 12)
E-Mail: ovi-info@arc.agric.za • Web site: www.arc.agric.za

Enquiries / Navrae

Ref. no. / Verw. nr.

MASTER STUDY FILE
TADP-S-15/01

Modelling the risk of foot-and-mouth disease virus outbreaks, assessing the effectiveness of vaccination and estimating the role of goats in FMD outbreaks continuation in South Africa.

Transboundry Animal Diseases Programme (High Containment Animal Facility)

ARC-Onderstepoort Veterinary Institute

Onderstepoort

0110

South Africa

On behalf of:
Dr Belinda Bilgnaut

ARC- Onderstepoort Veterinary Institute

Onderstepoort

0110

South Africa

Date of Commencement: 30 November 2015
Date of Completion: 17 December 2015

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Ref. no. / Verw. nr.

ARC-ONDERSTEPOORT VETERINARY INSTITUTE

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23 December 2015

Dr Belinda Blignaut
Transboundary Animal Diseases Programme
ARC- Onderstepoort Veterinary Institute

TADP-S-2015/01 STUDY FILE, Modelling the risk of foot-and-mouth disease virus outbreaks, assessing the effectiveness of vaccination and estimating the role of goats in FMD outbreaks continuation in South Africa. - Adaptation of virus in cattle and goats.

This letter serves to confirm that on 23 December 2015 the ARC-Onderstepoort Veterinary Institute (TADP High Containment Animal Facility) presented an authenticated copy of the Master Study File for Animal Experiment TADP-S-2015/01 to Dr Belinda Blignaut. All original study records will be retained by ARC for a minimum of two years from the end of the study.

It further serves to confirm that biological materials collected during the trial were transferred to the TADP Diagnostic Project for testing.

The ARC-Onderstepoort Veterinary Institute (TADP High Containment Animal Facility) considers this phase of the project to be completed. We would like to thank you for your support and looking forward to be of service to you again in the next phase of the clinical trial.

Yours sincerely,

Dr PB Mutowembwa
High Containment Animal Unit
Transboundary Animal Diseases Programme
ARC- Onderstepoort Veterinary Institute

Dr Belinda Blignaut
Researcher
Transboundary Animal Diseases Programme
ARC- Onderstepoort Veterinary Institute

Appendix B-2: Vaccine matching serum standardisation using liquid-phase blocking ELISA (sample)

LPBE 1

	SAT_1-ANTIGEN		SAT_2_ANTIGEN		SAT_3_ANTIGEN	
Samples	Titre (Rounded Percentage Inhibition)	Titre Actual	Titre (Roun	Titre Actual	Titre (Rou	Titre Actual
SAT_2_ BVI	2.5853	2.53784	1.7946	1.8412	2.2658	2.35260
SAT_2_CHA	1.9296	1.9670	1.9164	1.8973	2.0531	<1.3
SAT_1_CHA	2.7200	2.8077	1.7173	1.6172	1.9879	1.9605
SAT_1_BVI	2.5164	2.5480	1.7999	1.8385	2.4032	2.4717
LOW_POS_CONT	2.8044	>3.1	2.6148	2.6713	2.8439	>3.1
HIGH_POS_CONT	2.7466	2.8068	2.2514	2.1495	2.2099	2.2459
NEG_CONT	1.1762	<1.3	1.5654	<1.3	1.3864	<1.3
NEG_CONT	1.4308	<1.3	1.6465	<1.3	1.4757	<1.3

LPBE 2

	SAT_1-ANTIGEN		SAT_2_ANTIGEN		SAT_3_ANTIGEN	
Samples	Titre (Rounded Percentage Inhibition)	Titre Actual	Titre (Roun	Titre Actual	Titre (Rou	Titre Actual
SAT_1_ CHA_ORG	3.2	3.100	1.3	1.3	2.1	2.1
SAT_1_BVI_ORG	3	3	1.4	1.4	2.6	2.6
SAT_1_CHA_0,1	3.1	3.1	1.4	1.3	2	2
SAT_1_CHA_0,25	3	3.1	1.3	1.3	2	2
SAT_1_CHA_0,4	2.7	2.7	1.2	1.3	1.8	1.8
SAT_1_CHA_0,55	2.8	2.8	1.4	1.3	1.7	1.7
SAT_2_CHA_ORG	2	2	2	2	1.8	1.3
SAT_2_BVI_ORG	2.6	2.6	1.4	1.4	2.4	2.4
LOW_POS_CONT	2	2	1.5	1.5	1.7	1.7
HIGH_POS_CONT	3.1	3.1	1.9	1.9	2.5	2.5
NEG_CONT	1.2	1.3	1.2	1.3	1.2	1.3
NEG_CONT	1.2	1.3	1.2	1.3	1.2	1.3

Appendix B-3: Vaccine matching serum standardisation using VNT (sample)

DILUTION	Mean_Titre
0	1.404442934
0.5	2.407908372
0.25	2.00557368
0.125	1.806603676

DILUTION	PLATE_1
0	1.447
0.5	2.457
0.25	1.954
0.125	1.857

DILUTION	PLATE_2
0	1.361
0.5	2.357
0.25	2.056
0.125	1.755

DILUTION	Mean_Titre
0	1.531478917
0.5	2.656042111
0.25	2.17179291
0.125	1.870762915

DILUTION	PLATE_1
0	1.531
0.5	2.634
0.25	2.11
0.125	1.931

DILUTION	PLATE_2
0	1.531
0.5	2.677
0.25	2.232
0.125	1.809

DILUTION	Mean_Titre
0	1.500650467
0.5	2.3602037
0.25	2.283248208
0.125	1.698970004

DILUTION	PLATE_1
0	1.469
0.5	2.41
0.25	2.436
0.125	1.698

DILUTION	PLATE_2
0	1.531
0.5	2.309
0.25	2.13
0.125	1.698

ZIM_14_90	DILUTION	NATURAL SCALE
BVI	0	25.37715508
CH_0.5	0.5	255.8046129
CH_.25	0.25	101.2916581
CH_.125	0.125	64.06246951

ZIM_14_90	DILUTION	NATURAL SCALE_P1
BVI	0	27.9898132
CH_0.5	0.5	286.417797
CH_.25	0.25	89.94975815
CH_.125	0.125	71.9448978

ZIM_14_90	DILUTION	NATURAL SCALE_P2
BVI	0	22.96148648
CH_0.5	0.5	227.5097431
CH_.25	0.25	113.7627286
CH_.125	0.125	56.88529308

BOT_4_06	DILUTION	NATURAL SCALE
BVI	0	34
CH_0.5	0.5	452.9414973
CH_.25	0.25	148.5227255
CH_.125	0.125	74.26136277

BOT_4_06	DILUTION	NATURAL SCALE_P1
BVI	0	33.96252726
CH_0.5	0.5	430.5266105
CH_.25	0.25	128.8249552
CH_.125	0.125	85.3100114

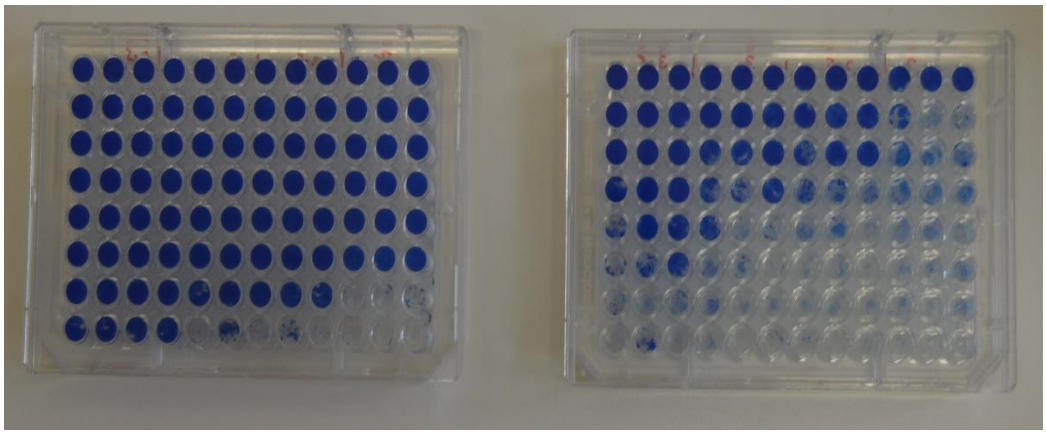
BOT_4_06	DILUTION	NATURAL SCALE_P2
BVI	0	33.96252726
CH_0.5	0.5	475.3352259
CH_.25	0.25	170.6082389
CH_.125	0.125	64.41692655

KNP_19_89	DILUTION	NATURAL SCALE
BVI	0	31.67017524
CH_0.5	0.5	229.1942408
CH_.25	0.25	191.9765611
CH_.125	0.125	50

KNP_19_89	DILUTION	NATURAL SCALE_P1
BVI	0	29.44421634
CH_0.5	0.5	257.0395783
CH_.25	0.25	272.8977783
CH_.125	0.125	49.88844875

KNP_19_89	DILUTION	NATURAL SCALE_P2
BVI	0	33.96252726
CH_0.5	0.5	203.7042078
CH_.25	0.25	134.8962883
CH_.125	0.125	49.88844875

Appendix B-4: Image of VNT plate result



Appendix C

Appendix C-1: Weighting questionnaire of risk factors for FMD occurrence

THE SPATIAL RISK OF FMD *OCCURRENCE* WITHIN THE PROTECTION ZONE WITH VACCINATION OF SOUTH AFRICA
RISK FACTOR WEIGHTING

Thank you for your participation. In our study, we are assessing the risk of FMD outbreak *OCCURRENCE* in South Africa.

FMD Occurrence is defined as the detection of an FMD outbreak within a village or a dip-tank

A set of risk factors based on the literature and available data are listed below.

We require your expertise to define the appropriate weight (=importance) for each selected risk factor.

Risk of SAT1 & SAT2 FMD occurrence	**Associated hypothesis**
Cattle population	Increasing cattle density increases the likelihood of FMD outbreak occurrence.
Proximity to a game reserve	Shorter distance to a game reserve fence increases the likelihood of FMD outbreak occurrence.
Human population	Higher human density increases the likelihood of FMD outbreak occurrence.
Proximity to a road network	Closer proximity to a road network increases the likelihood of FMD outbreak occurrence.
Proximity to rivers	Closer proximity to rivers increases the likelihood of FMD outbreak occurrence.
Vaccine matching	Poorer vaccine matching increases the likelihood of FMD outbreak occurrence.
Vaccination coverage	Lower vaccination coverage increases the likelihood of FMD outbreak occurrence.
Vaccination interval	Longer vaccination intervals increase the likelihood of FMD outbreak occurrence.
Cattle inspection	Lower inspection effectiveness increases the likelihood of FMD outbreak occurrence.
Permitted cattle movement into a village/location	Higher number of cattle movements into a village increases the likelihood of FMD outbreak occurrence.
Permitted cattle movement outside a village/location	Higher number of cattle movements leaving a village increases the likelihood of FMD outbreak occurrence (within the village sending the cattle out).

Please select an option from each drop-down list that corresponds to the risk of *FMD OCCURRENCE* related to each factor compared to all others. Comparisons are to be made between each Row Factor (BLUE) to the corresponding Column Factor (ORANGE) for each cell in the table.

(Comparing the *BLUE* shaded factors to the *ORANGE* shaded factors).

	Cattle population	Proximity to a game reserve	Human population	Proximity to a road network	Proximity to rivers	Vaccine	Vaccination coverage	Vaccination interval	Cattle inspection	Permitted cattle movement into a	Permitted cattle movement outside a
Cattle population	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Proximity to a game reserve	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Human population	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Proximity to a road network	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Proximity to rivers	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Vaccine matching	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Vaccination coverage	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Vaccination interval	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Cattle inspection	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Permitted cattle movement into a village/location	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Permitted cattle movement outside a village/location	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1

Please use the scale provided to identify your choice

More Important				Equivalent	Less Important			
Extremely	Very	Strongly	Moderately		Moderately	Strongly	Very	Extremely
16 : 1	8 : 1	4 : 1	2 : 1	1 : 1	1 : 2	1 : 4	1 : 8	1 : 16

Appendix C-2: Weighting questionnaire of risk factors for FMD spread

THE SPATIAL RISK OF FMD *SPREAD* WITHIN THE PROTECTION ZONE WITH VACCINATION OF SOUTH AFRICA
RISK FACTOR WEIGHTING

Thank you for your participation. In our study, we are assessing the risk of FMD outbreak *SPREAD* in South Africa.

FMD spread is defined as the transmission or movement of FMD outbreak to a secondary village or a dip-tank.

A set of risk factors based on the literature and available data are listed below.

We require your expertise to define the appropriate weight (=importance) for each selected risk factor.

Risk of SAT1 & SAT2 FMD spread	**Associated hypothesis**
Cattle population	Increasing cattle density increases the likelihood of local FMD outbreak spread.
Proximity to a game reserve	Shorter distance to a game reserve fence increases the likelihood of FMD outbreak spread.
Human population	Higher human density increases the likelihood of FMD outbreak spread.
Proximity to a road network	Closer proximity to a road network increases the likelihood of FMD outbreak spread.
Proximity to rivers	Closer proximity to rivers increases the likelihood of FMD outbreak spread.
Vaccine matching	Poorer vaccine matching increases the likelihood of FMD outbreak spread.
Vaccination coverage	Lower vaccination coverage increases the likelihood of FMD outbreak spread.
Vaccination interval	Longer vaccination intervals increase the likelihood of FMD outbreak spread.
Cattle inspection	Lower inspection effectiveness increases the likelihood of FMD outbreak spread.
Permitted cattle movement into a	Higher number of cattle movements into a village increases the likelihood of FMD outbreak spread (from the receiving village to a new village).
Permitted cattle movement outside a village/location	Higher number of cattle movements leaving a village increases the likelihood of FMD outbreak spread (to a new village).

Please select an option from each drop-down list that corresponds to the risk of *FMD SPREAD* related to each factor compared to all others. Comparisons are to be made between each Row Factor (BLUE) to the corresponding Column Factor (ORANGE) for each cell in the table. (Comparing the *BLUE* shaded factors to the *ORANGE* shaded factors).

	Cattle population	Proximity to a game reserve	Human population	Proximity to a road network	Proximity to rivers	Vaccine matching	Vaccination coverage	Vaccination interval	Cattle inspection	Permitted cattle movement into a	Permitted cattle movement outside a
Cattle population	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Proximity to a game reserve	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Human population	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Proximity to a road network	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Proximity to rivers	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Vaccine matching	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Vaccination coverage	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Vaccination interval	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Cattle inspection	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Permitted cattle movement into a village/location	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1
Permitted cattle movement outside a village/location	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1	16 : 1

Please use the scale provided to identify your choice

More Important				Equivalent	Less Important			
Extremely	Very	Strongly	Moderately		Moderately	Strongly	Very	Extremely
16 : 1	8 : 1	4 : 1	2 : 1	1 : 1	1 : 2	1 : 4	1 : 8	1 : 16

Appendix C-3: Risk factors and expert opinion elicitation

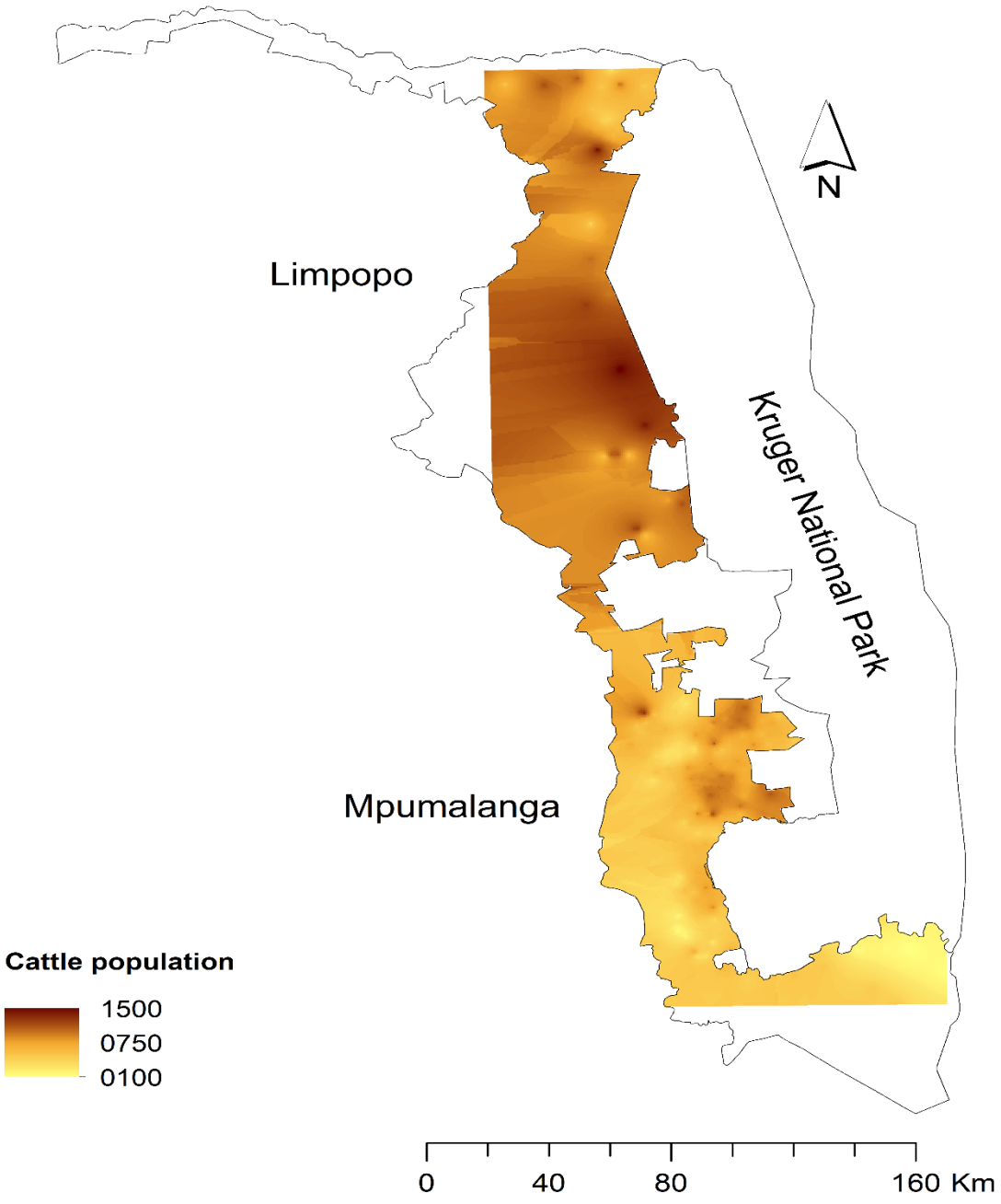
Standardized potential risk factor for SAT1 and SAT2 FMD occurrence and spread

Potential risk factor for SAT1 and SAT2 FMD occurrence and spread	Mean	SD
Permitted cattle movement outside a village/location	4.96	5.34
Proximity to a road network	9.65	10.27
Proximity to rivers	16.18	11.49
Human population (density)	1312.77	1023.37
Cattle inspection (proportion)	0.42	0.20
Permitted cattle movement into a village/location	2.69	2.77
Vaccination coverage (vaccination proportion)	0.09	0.07
Vaccination interval	10.56	10.23
Dip-tanks weighted average for a combined SAT1 and SAT2 FMD vaccine matching	0.12	0.03
Cattle population	666.84	351.87
Proximity to a game reserve	8.60	7.21

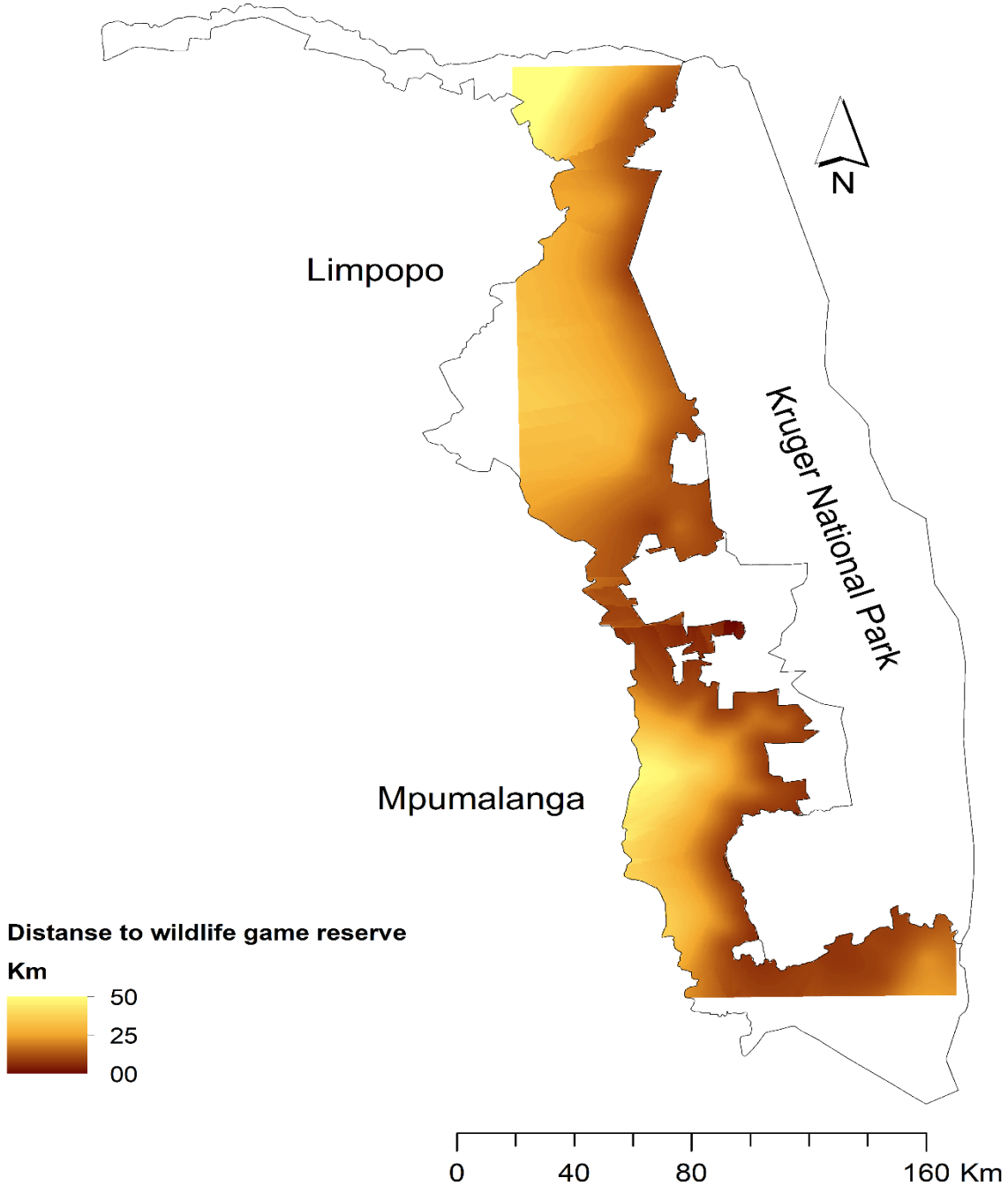
Pairwise FMD risk factor's wights

Potential risk factor for SAT1 and SAT2 FMD occurrence and spread	FMD occurrence	FMD Spread
Permitted cattle movement outside a village/location	-2.30	-2.44
Proximity to a road network	-0.61	-0.17
Proximity to rivers	-0.60	-0.55
Human population (density)	-0.51	-0.04
Cattle inspection (proportion)	-0.28	-0.72
Permitted cattle movement into a village/location	0.16	0.21
Vaccination coverage (vaccination proportion)	0.70	0.70
Vaccination interval	0.73	0.99
Dip-tanks weighted average for a combined SAT1 and SAT2 FMD vaccine matching	0.74	0.63
Cattle population	0.75	1.10
Proximity to a game reserve	1.21	0.28

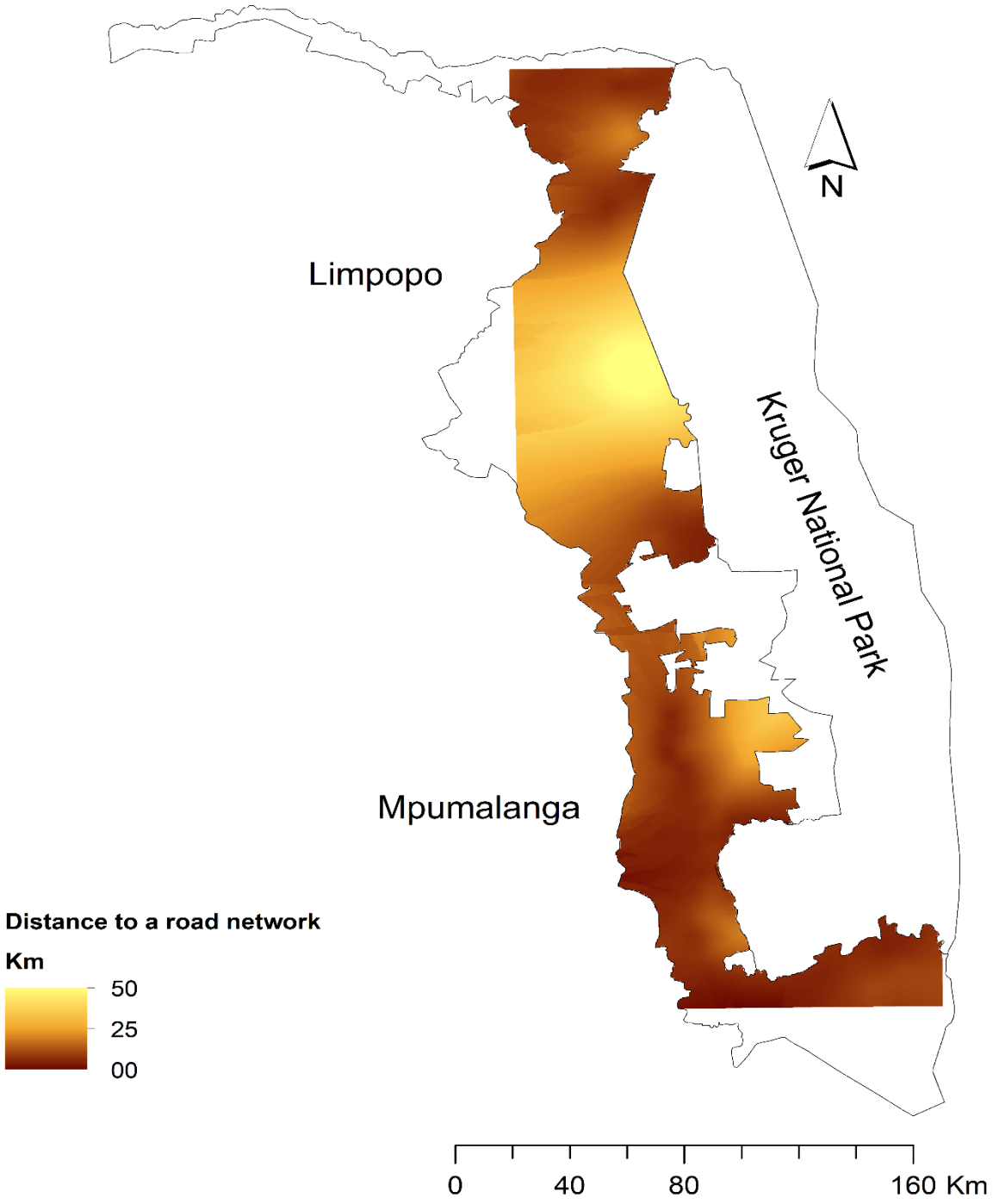
Appendix C-4 -C-16: Empirical Bayesian kriging for individual risk factors for FMD occurrence and spread in the Protection vaccination zone of South Africa (2007-2016)



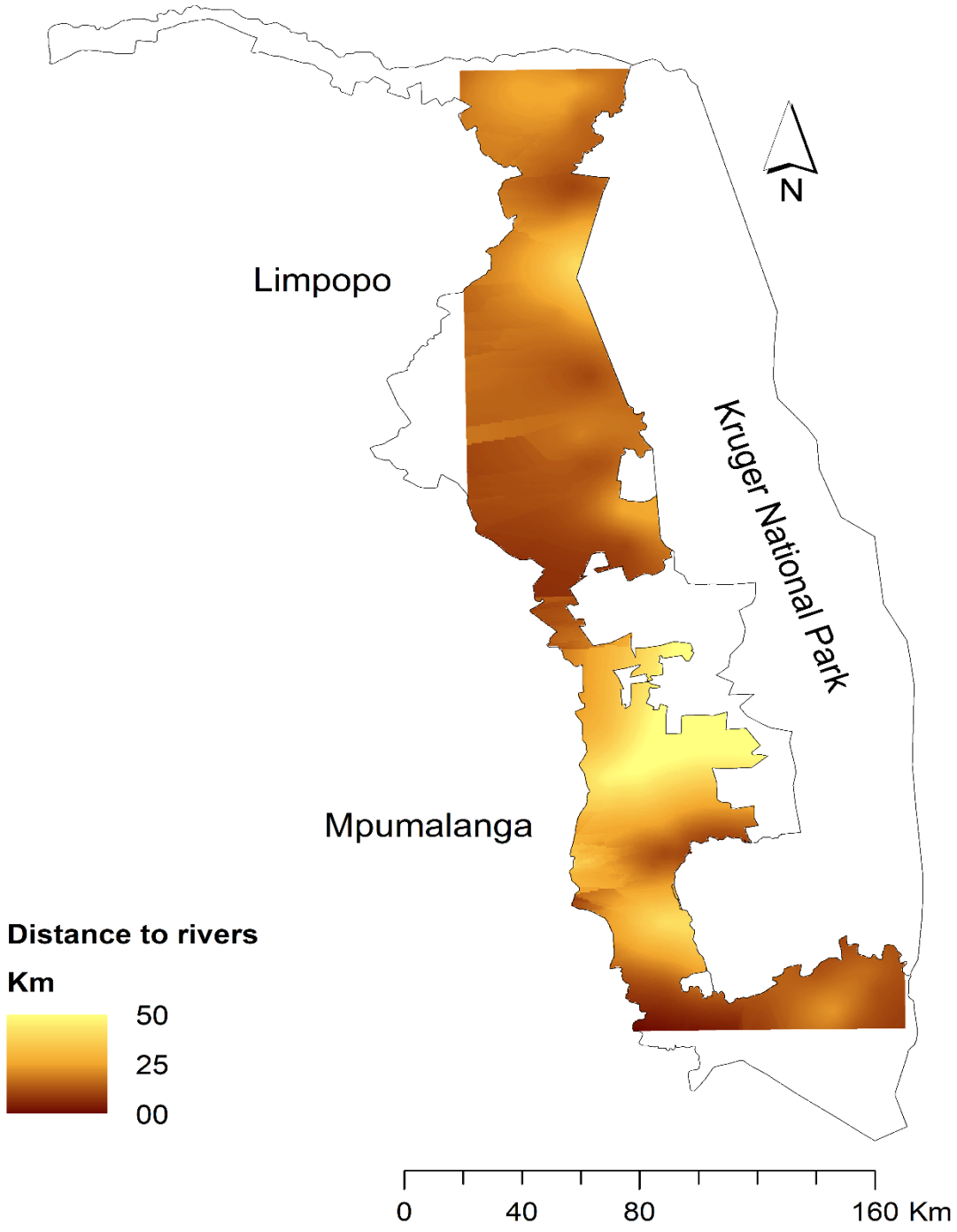
Appendix C-4: Empirical Bayesian kriging (cell size 5) for cattle population in the FMD Protection vaccination zone of South Africa (2007-2016).



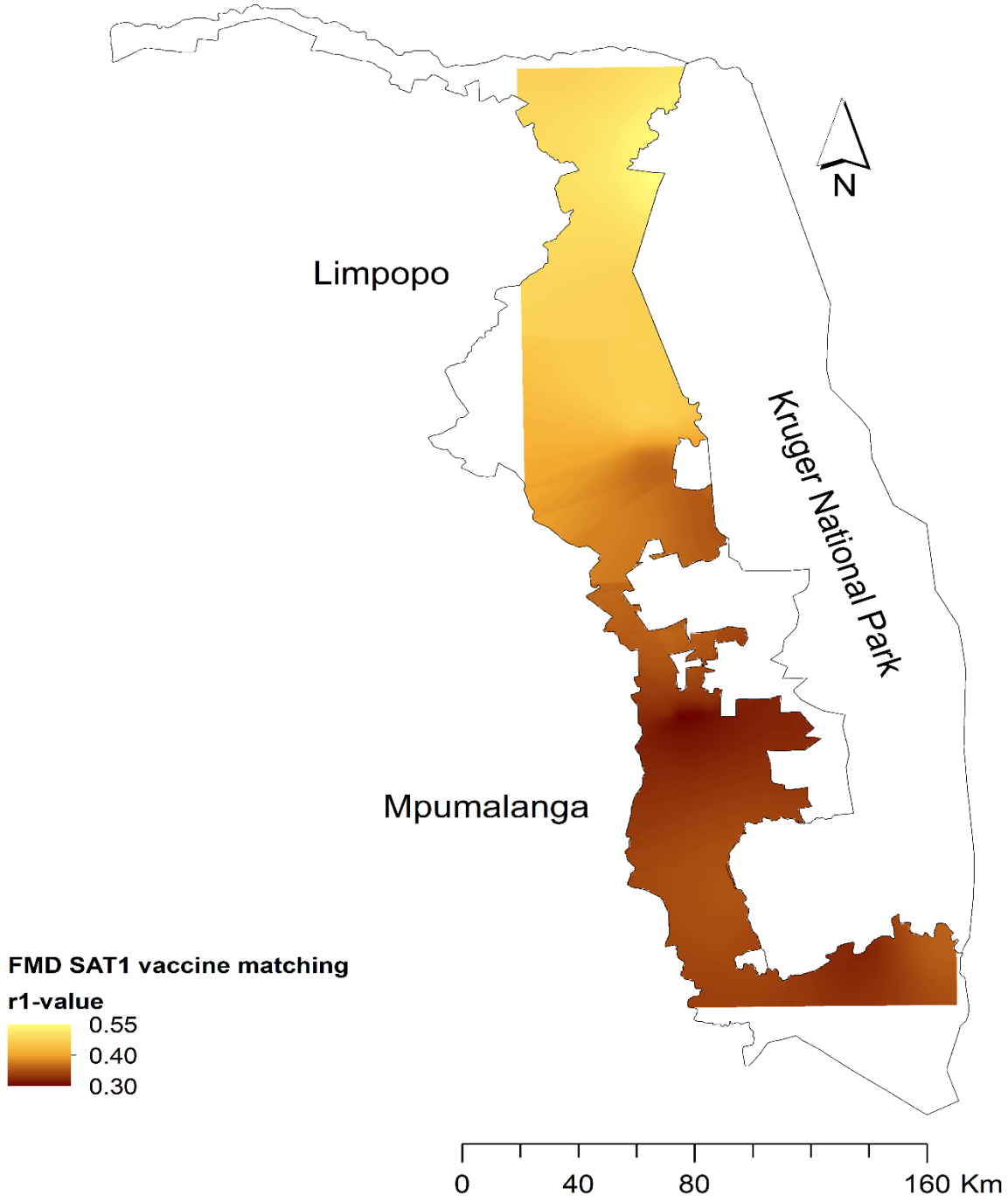
Appendix C-5: Empirical Bayesian kriging (cell size 5) for proximity of dip-tanks in the FMD Protection zone with vaccination of South Africa to wildlife nature reserves.



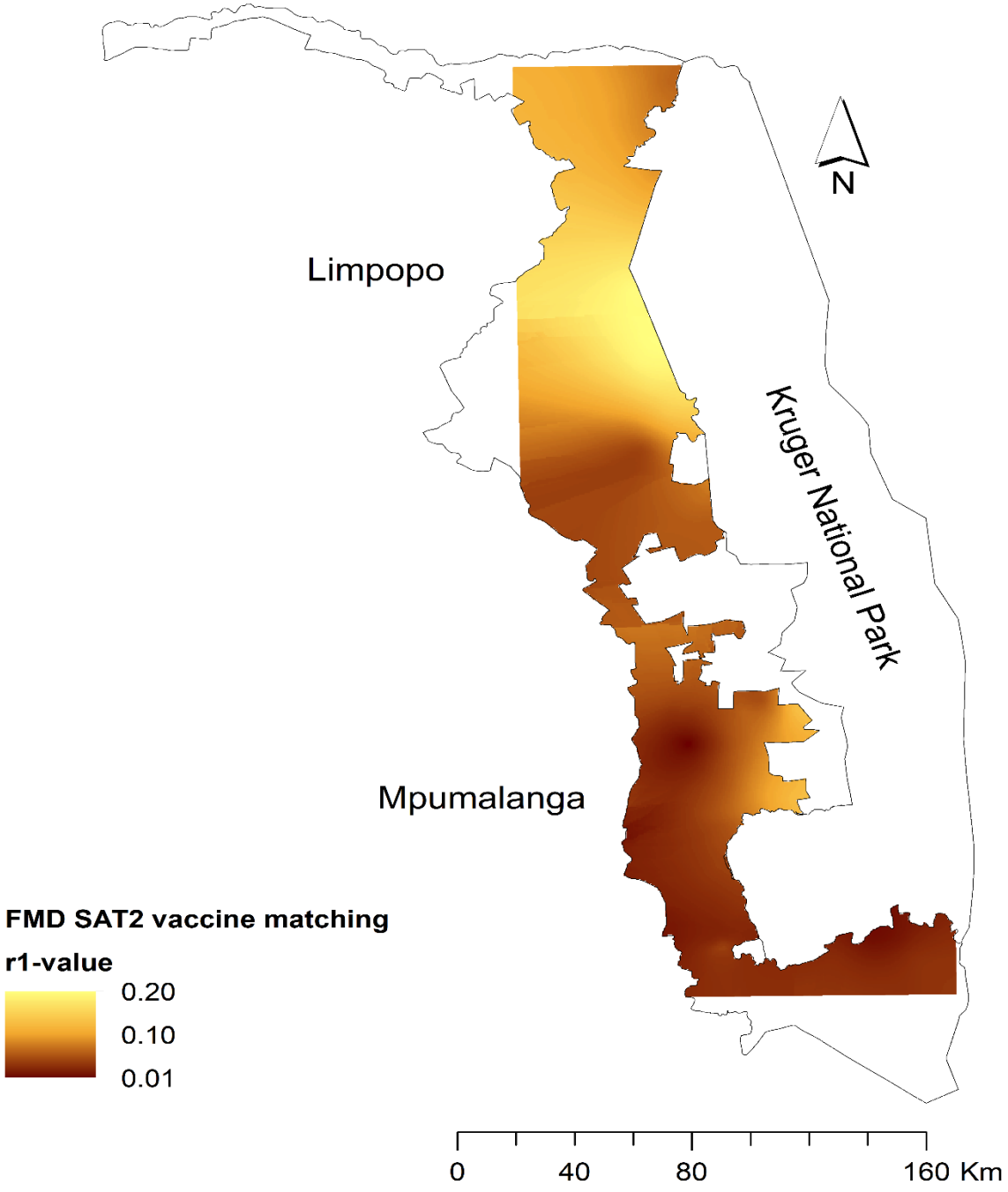
Appendix C-6: Empirical Bayesian kriging (cell size 5) for proximity of dip-tanks in the FMD Protection zone with vaccination of South Africa to road networks



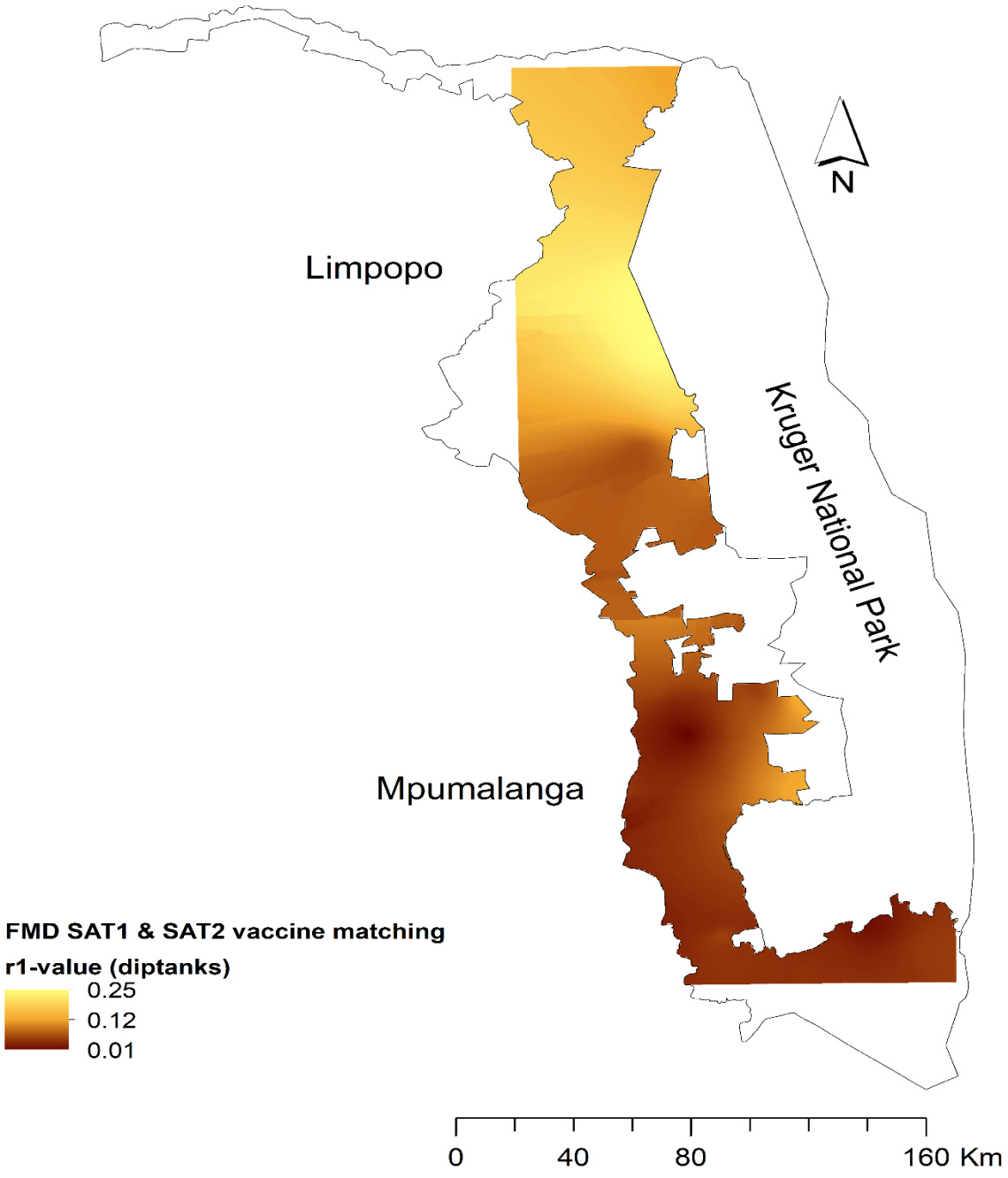
Appendix C-7: Empirical Bayesian kriging (cell size 5) for proximity of dip-tanks in the FMD Protection zone with vaccination of South Africa to rivers



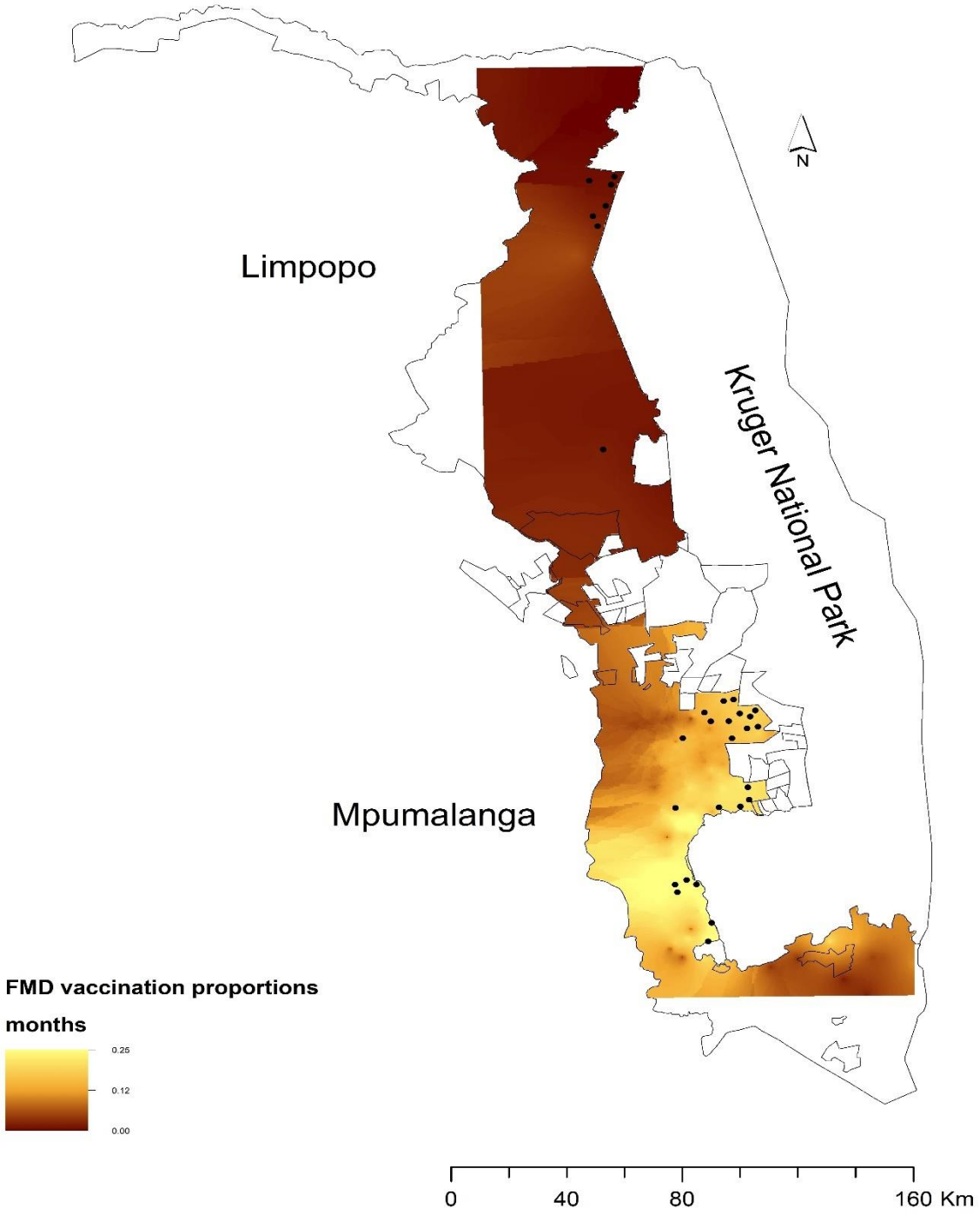
Appendix C-8: Empirical Bayesian kriging (cell size 5) for SAT1 FMDV vaccine matching in the FMD protection zone with vaccination of South Africa (2007-2016).



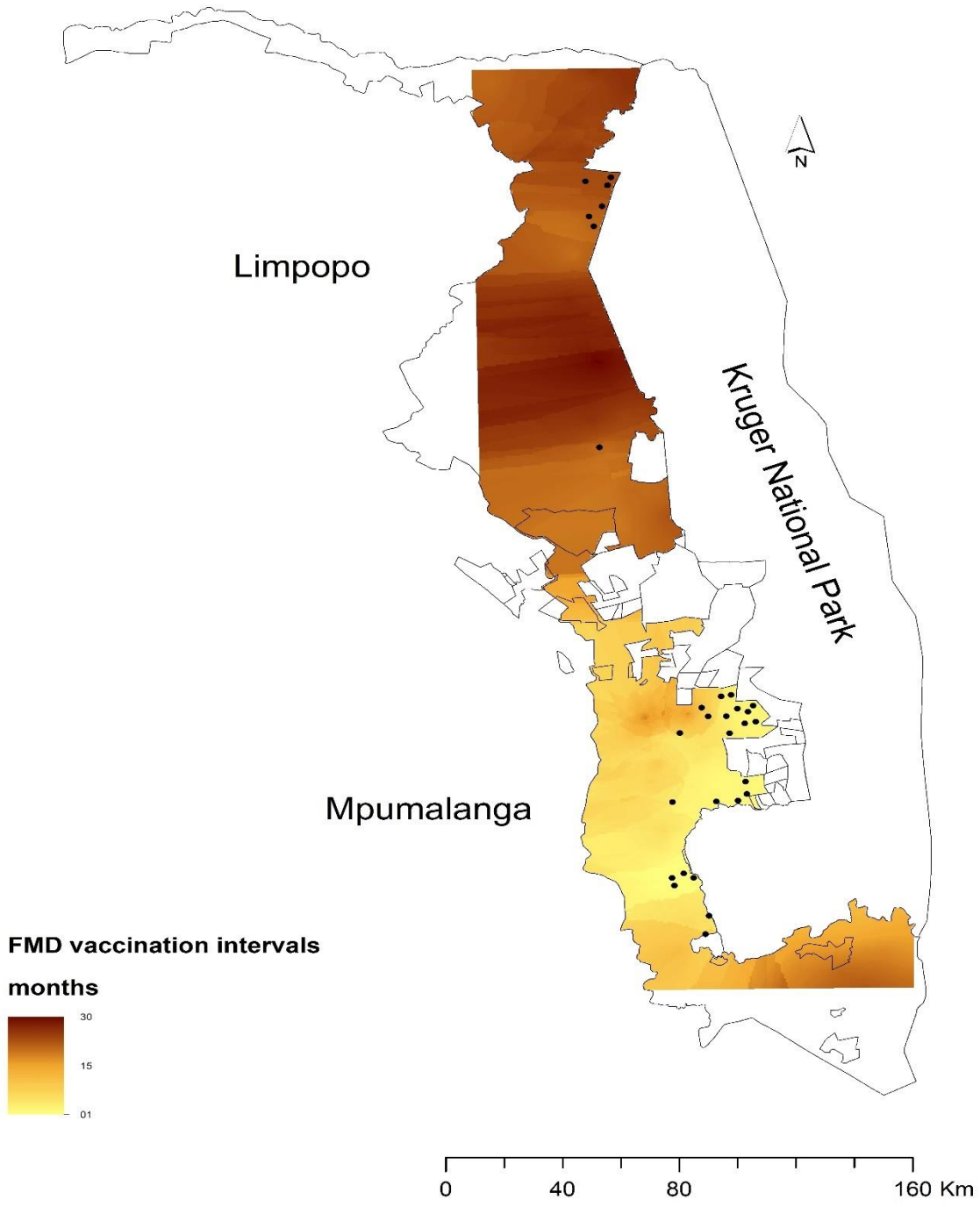
Appendix C-9: Empirical Bayesian kriging (cell size 5) for SAT2 FMDV vaccine matching in the FMD protection zone with vaccination of South Africa (2007-2016).



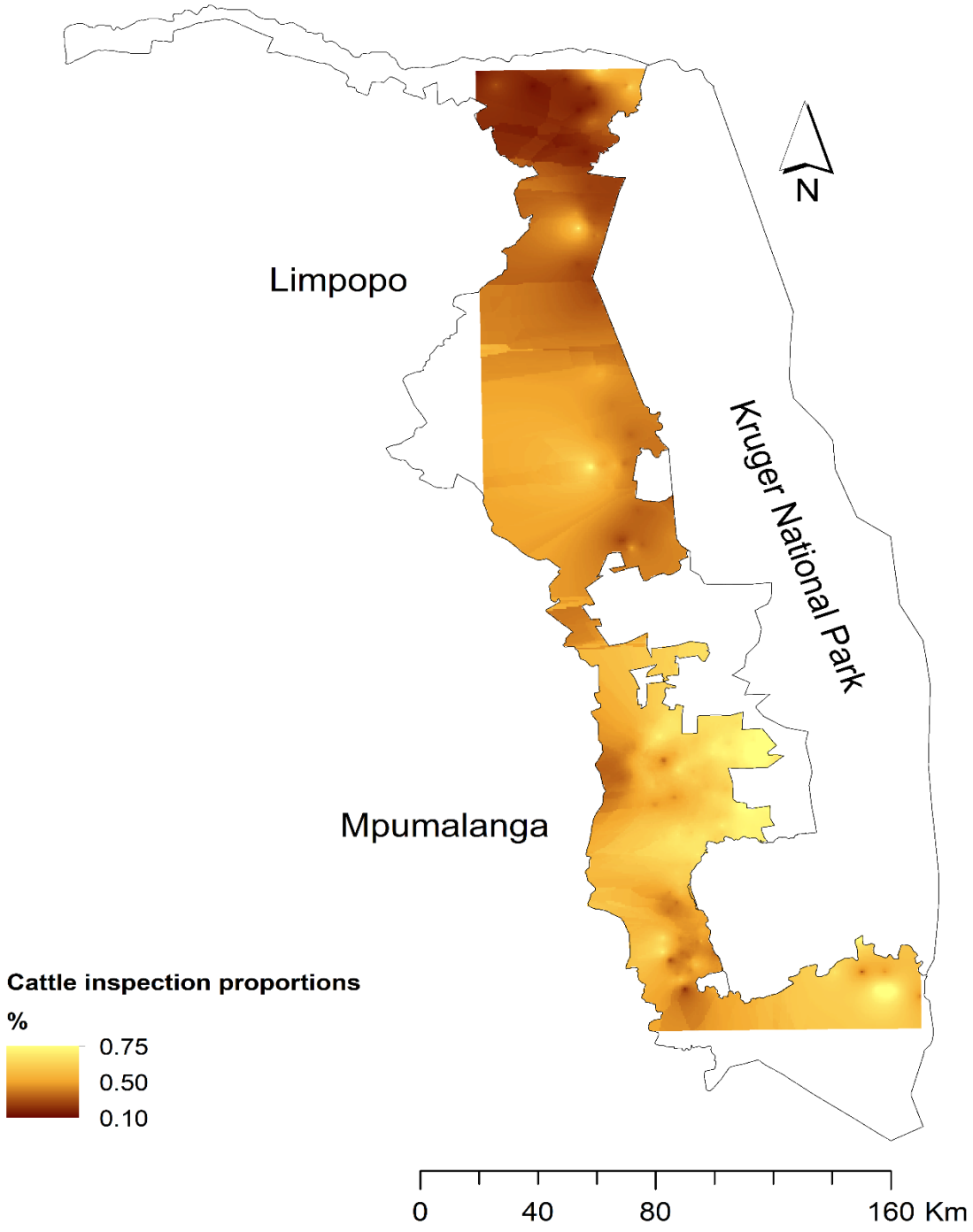
Appendix C-10: Empirical Bayesian kriging (cell size 5) for weighted SAT1 and SAT2 FMDV vaccine matching weighted by affected dip-tanks in the FMD protection zone with vaccination of South Africa (2007-2016).



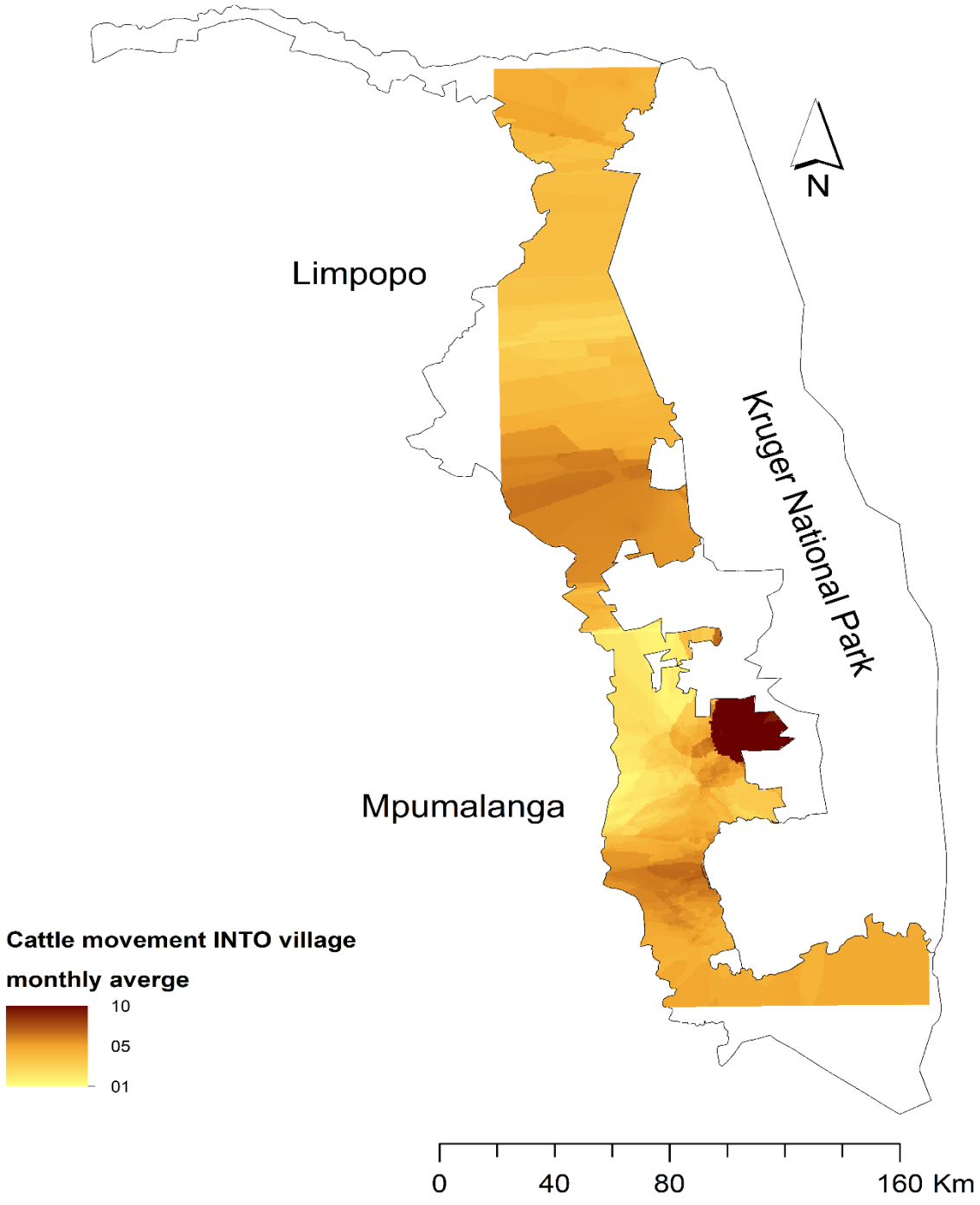
Appendix C-11: Empirical Bayesian kriging (cell size 5) for vaccination proportions in the FMD protection zone with vaccination of South Africa (2007-2016).



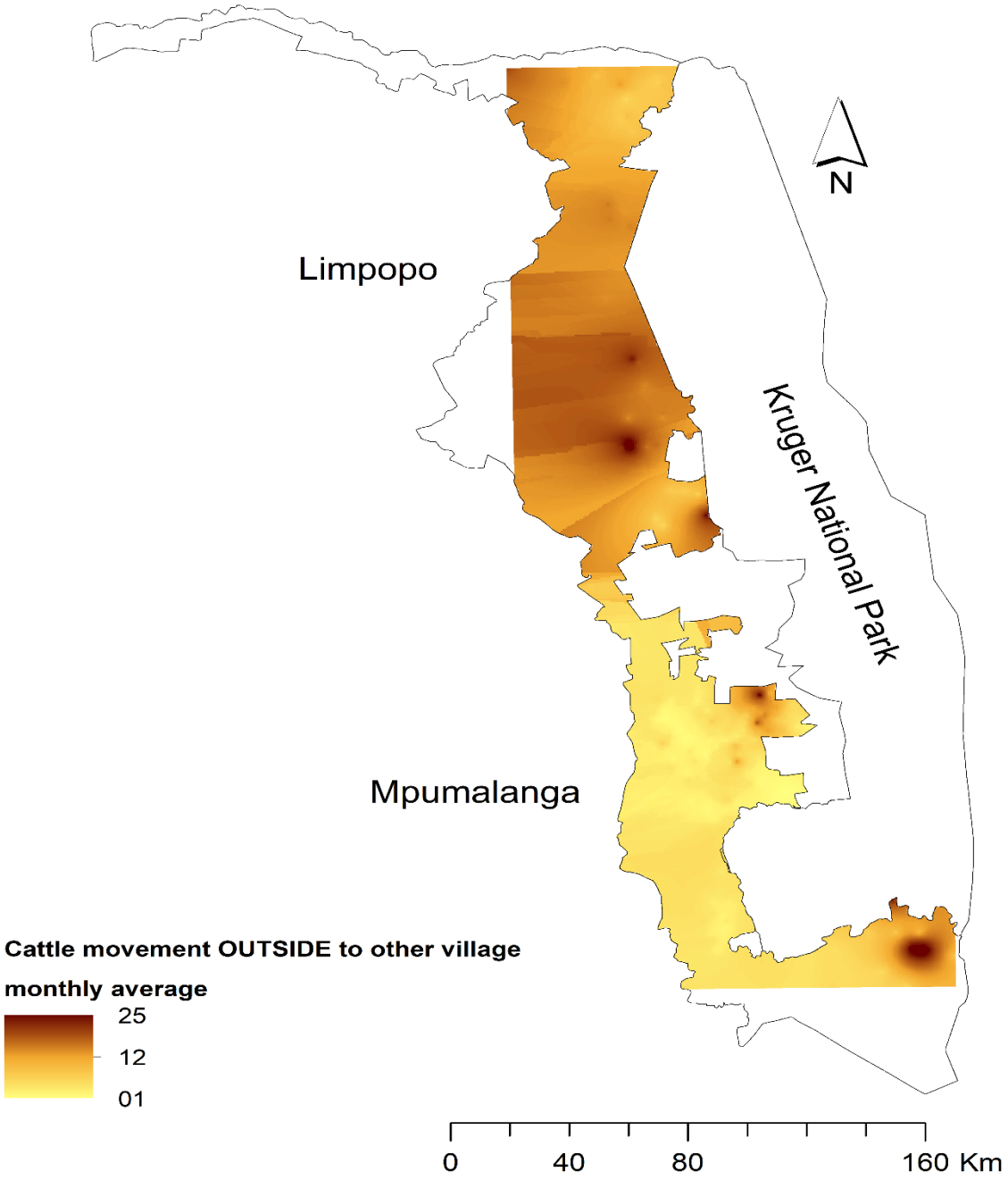
Appendix C-12: Empirical Bayesian kriging (cell size 5) for vaccination intervals in the FMD Protection zone with vaccination of South Africa (2007-2016).



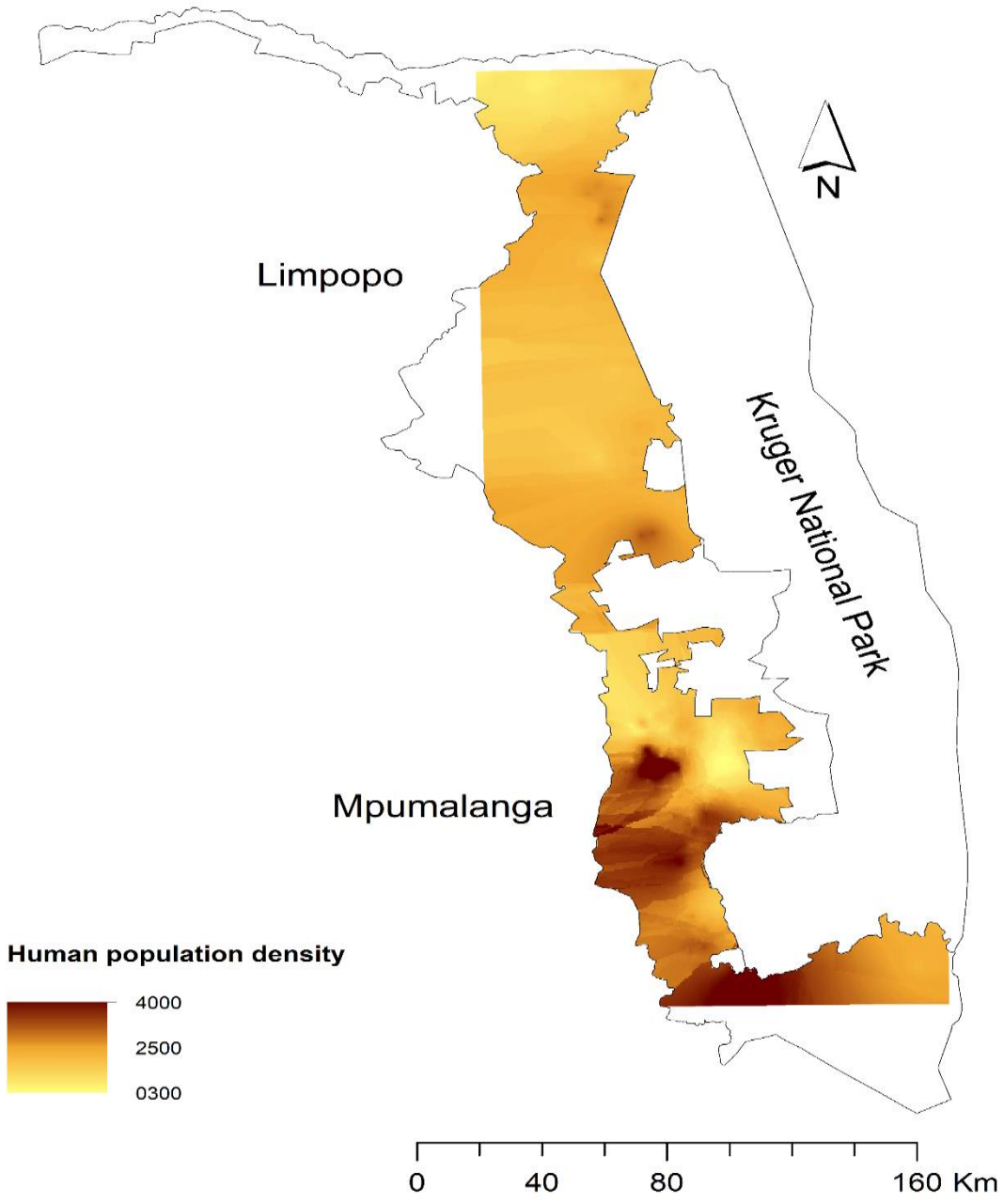
Appendix C-13: Empirical Bayesian kriging (cell size 5) for cattle FMD inspections in the FMD Protection zone with vaccination of South Africa (2007-2016).



Appendix C-14: Empirical Bayesian kriging (cell size 5) for cattle movement into a dip-tank (village) in the FMD Protection zone with vaccination of South Africa (2007-2016).



Appendix C-15: Empirical Bayesian kriging (cell size 5) for cattle movement outside a dip-tank to another (village) in the FMD Protection zone with vaccination of South Africa (2007-2016).



Appendix C-16: Empirical Bayesian kriging (cell size 5) for human population density in the FMD Protection zone with vaccination of South Africa (2011).

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Research

26-Feb-2015

Approval Certificate

New Application

Ethics Reference No.: V005-15

Title: Short Title: The role of goats and vaccination in disease outbreaks of Foot-and-mouth Disease in South Africa. Title: Modelling the risk of Foot-and-mouth Disease virus outbreaks, assessing the effectiveness of vaccination and estimating the role of goats in FMD outbreaks continuation in South Africa.

Dear Dr Mohamed Sirdar Issa

The **New Application** as supported by documents specified in your application or your research received, was approved by the Animal Ethics Committee on the 23-Feb-2015.

Please note the following about your ethics approval:

- Ethics Approval is valid from *26-Feb-2015* to *25-Feb-2016*.
- Please remember to use your protocol number (V005-15) on any documents or correspondence with the Animal Ethics Committee regarding your research.
- Please note that the Animal Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:

- The ethics approval is conditional on the receipt of an annual (after 12 months) written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

With regards

Dr Daan Verwoerd

Chair Animals Ethics Committee, University of Pretoria