Efficacy of foot-and-mouth disease vaccination in goats and movement patterns of livestock amongst smallholder farmers in Mnisi, Bushbuckridge, South Africa

David Dazhia Lazarus

Submitted in partial fulfilment of the requirements for the degree
Doctor of Philosophy
Veterinary Science
Production Animal Studies

Faculty of Veterinary Science
University of Pretoria

September 2020

DEDICATION

This thesis is dedicated to the LORD Almighty and my late father Mr. **Lazarus Dazhia, Skam** who inspired my dream for a career in veterinary science at the early stage of my life. May his gentle soul rest in peace.

DECLARATION

I declare that all work presented in this thesis has been carried out by myself except where acknowledged. This thesis has not been submitted in any form for another degree or diploma at any university or institution of higher learning.

D D Lazarus

……

ACKNOWLEDGEMENTS

The author wishes to thank the following persons and institutions:

Firstly, I would like to thank my supervisors and collaborators: Prof Geoffrey T. Fosgate, Dr Pamela A. Opperman and Dr Livio Heath for their kind supervision, support and direction during the study. Their various strengths and dedication toward the success of this study is well appreciated.

Ms Daleen Anderson, Sr Igrid de Goede and Ms Margaret Nthabiseng Louw of the Department of Production Animal Studies, for being helpful and out there for me all through the study period. Dr Rebone Moerane, Head of Department of Production Animal Studies for his usual supports. Ms Marie Watson-Kriek for her usual logistic and technical supports for my project.

Dr Richard E J Burroughs for his fatherly and pastoral care. Prof Leith Meyer (Director: Centre for Veterinary Wildlife Studies) for providing me will all the necessary logistic support. Prof Darrel Abernethy for his wise counsels. Prof Gerry Swan (Director: Exotic Leather Research Centre) for equally proving me with logistic support. Nobri Booyzen, Yvette Van Den Berg, and Christoff Truter of the Exotic Leather Research Centre for their kindness and support. Eddy Diasonua a former ICT specialist at the ELRC for his supports and encouragements during the programme. Prof Alan Guthrie (Director: Equine Research Centre) for always been there for me and encouraging me all through the programme. Dr Musafiri Karama is well acknowledged for his pastoral counsels, guidance and being a prayer partner during the study.

Jeanette Wentzel, Johan Gouws and his caring wife Makkie at Hans Hoheisen Wildlife Research Station (HHWRS) Orpen, Kruger National Park for making the Station a second home to me.

Dr Ilana van Wyk of the Mnisi Community Programme (MCP) for her technical and logistic

supports during fieldwork at the Mnisi Tribal Authority of Bushbuckridge, Mpumalanga. Her husband Mr J P van Wyk for always there to assist where there is need.

Dr Oupa Rikhotso of the Mpumalanga Veterinary Services and his teams for making available resources for the fieldwork in Mnisi. Mr Jerry Ndlozi, Mr Thabo Lekhuleni and Mr Solly Mokone for their logistic supports during fieldwork in Mnisi.

We acknowledge all smallholder farmers and the dip tank Committees for Animal Health Wards Bushbuckridge (B1-B3) for participation in the livestock movement study. The 3 Environmental Assistants for assisting with information translation and data collection during the fieldwork.

Dr Louise Biggs of the Hluvukani Animal Centre and her team and final year clinical students on rotations for their regular support and assistance. Ms Addlove Sekgobela, Ms Violet Mathebula and Ms Dimakatso Julia Sithole of the Mnisi Community Programme for their assistance with the administration of the questionnaire and facilitation of the focus group discussion.

Ms Erika Kirkbride, Ronel Taljaard and Kerstin Malan of the Administrative unit of the TAD/OVR for their logistic supports and assistance. Dr P B Mutowembwa and R M Thapelo of the BSL-3 animal facility for their assistance with animal trials. All animal handlers in the facility are well acknowledged. Ms Danica van der Merwe, Juanita van Herdeen and LaToya Seoke for their assistance with sample analysis. Henry Harris, Hanlie Jones, Robert Mkhwanazi, Keneiloe Montsu, Refiloe Malesa of the Diagnostics section of TAD/OVR for their assistance with sample analysis. Dr Nefefe Tshifhiwa of the Immunology section for his supports and guidance during the study. Faith Peta is well acknowledged for her work on development of the vaccine and supports during the study.

Lina Lakah and Fozia Solomon of the Client Service Centre for their special supports.

Cassius Mngomezulu and Leonie Johnson of the Postgraduate Administration for their administrative supports.

My family: Ms Mary Lazarus, Engr Yakubu L Dazhia, Rev Samuel Lazarus, my mum Ms Saraya Lazarus, friends and colleagues; Dr & Dr (Ms) Francis Kolo, Dr & Ms Okey Ndumnego, Dr & Ms M S Liman, Drs Yusuf B Ngoshe, Solomon J Thliza, Samuel Ogundare, Sunday Ochai, Musa Zakariya and Hassan Ibrahim for their encouragement, interest and believe in me. A special thanks to my wife Ms Pati David Lazarus and my daughter Tamara David for their love, sacrifices, encouragement and supports.

The author also wishes to express his sincere gratitude to:

The National Research Foundation of South Africa for providing funding for the study.

The University of Pretoria Doctoral Support Programme for providing funding.

The Agricultural Research Council of South Africa for provision of research supports.

The Peace Parks Foundation for supplementary funding.

The Federal Republic of Nigeria and the National Veterinary Research Institute, Vom Nigeria for granting me the opportunity to further my career.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

α	alpha
AEC	Animal ethics committee
ANOVA	Analysis of variance
β	beta
BEI	Binary ethyleneimine
BHK	Baby hamster kidney
BOT	Botswana
BRVB	Bovine rhinitis virus B
BSL-3	Biosafety level -3
BTY	Bovine thyroid
BVI	Botswana Vaccine Institute
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CI	Confidence interval
COVID-19	Coronavirus-2019
CPE	Cytopathic effect
δ	delta
°C	degrees Celsius
DAFF	Department of Agriculture, Forestry and Fisheries
DC	Dendritic cell
DIVA	Differentiating infected from vaccinated animals
DNA	Deoxyribonucleic acid
DPC	Days post challenge
DPV	Days post vaccination
eIF	eukaryotic initiation factor
ELISA	Enzyme-linked immunosorbent assay

FAO	Food and Agriculture Organisation
FMD PZ	Foot-and-mouth disease protection zone
FMD	Foot-and-mouth disease
FMDV	Foot-and-mouth disease virus
γ	Gamma
GF-TADs	Global Framework for Transboundary Animal Diseases
GPS	Global positioning system
GTF	Geoffrey Theodore Fosgate
h	Hour
HPAI	Highly pathogenic avian influenza
IB-RS 2	Instituto Biologico Rim Suino-2
ID	Identification
e.g.	Exempli gratia (for example)
et al.,	et alia (and others)
etc	et cetera
Ig	Immunoglobulin
IL	Interleukin
INF	Interferon
IQR	Interquartile range
KNP	Kruger National Park
LAK	Lymphokine activated killer
LFBK	Adherent foetal porcine cell line
LFD	Lateral flow device
LPBE	Liquid phase blocking ELISA
L^{pro}	Leader proteinase
MAb	Monoclonal antibody
MHC	Major histocompatibility complex

MTA	Mnisi Tribal Authority
MVPK-1	Mengeling Vaughn Porcine Kidney-1
NFkB	Nuclear factor kappa B
NK	Natural killer
NSP	Non-structural protein
OD	Optical density
OIE	Office International des Epizooties
ORF	Open reading frame
OVI	Onderstepoort Vaccine Institute
OVR	Onderstepoort Veterinary Research
PBMC	Peripheral blood mononuclear cell
PCP-FMD	Progressive Control Programme for Foot-and-mouth disease
PCR	Polymerase chain reaction
PD_{50}	50% protective dose
PI	Percentage inhibition
PRR	Pattern recognizing receptors
PRRS	Porcine reproductive and respiratory syndrome
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute-1640
RT-LAMP	Reverse transcription loop-mediated isothermal amplification
RT-PCR	Reverse-transcriptase polymerase chain reaction
SADC	Southern African Development Community
SAR	South Africa Republic
SAT	Southern African Territories
SD	Standard deviation
SPCE	Solid phase competition ELISA

TAD	Transboundary Animal Disease
$TCID_{50}$	50% tissue culture infective dose
TFCA	Transfrontier conservation area
Th	Helper T lymphocyte
TNF	Tissue necrosis factor
UK	United Kingdom
UTR	Untranslated region
UVC	Unvaccinated control
VLP	Virus-like particles
VNT	Virus neutralization test
VP	Viral protein
VPN	Veterinary Procedural Notice
ZAF	South Africa
ZZ-R 127	Foetal goat tongue cell line

ABSTRACT

Efficacy of foot-and-mouth disease vaccination in goats and movement patterns of livestock amongst smallholder farmers in Mnisi, Bushbuckridge, South Africa

Candidate: David Dazhia Lazarus

Promoter: Professor Geoffrey T. Fosgate

Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

Co-promoter: Dr Pamela A. Opperman

Agricultural Research Council, Transboundary Animal Diseases Programme, Onderstepoort Veterinary Research

Abstract

Foot-and-mouth disease (FMD) is a transboundary animal disease that has a major impact on livestock production, regional and international trade and livelihoods of smallholder farmers in endemic settings. The disease is caused by infection with foot-and-mouth disease virus (FMDV), a single stranded RNA virus that affects cloven-hoofed livestock and wildlife. Goats are susceptible to infection with FMDV, but their role in the epidemiology of the disease and response to vaccination is poorly understood. In southern Africa, FMDV serotypes Southern African Territories (SAT) 1, 2 and 3 are known to be endemic. In this study, we described the clinical presentation of FMDV SAT1 in goats, the efficacy of an oil emulsion FMD vaccine against heterologous challenge with a pool of field SAT1 FMDVs and described the patterns of livestock movements among smallholder farmers within a section of the FMD protection zone of Mpumalanga Province, South Africa.

For this study, forty FMD sero-negative goats (6-12 months of age) of mixed sexes obtained from the FMD free zone of the country were randomly allocated to one of five treatment groups: G1 full cattle dose (2 ml), G2 (0.67 ml), G3 (0.33 ml), G4 (0.16 ml) or G5 (unvaccinated placebo) control. Goats were vaccinated with an inactivated FMD vaccine containing FMDV serotype SAT1 on day 0 and revaccinated at day 20 post vaccination. Thereafter, thirty-four goats were challenged by tongue inoculation at day 41 post-vaccination using $10^{4.57}$ 50% tissue culture infective dose ($TCID_{50}$) of a FMDV SAT1 pool. Animals were examined daily, and clinical signs were scored. Rectal temperatures were measured daily, with temperatures ≥40°C defined as fever. Clinical specimens (nasal, oral and rectal swabs) were collected on days 0, 2, 4 and 6 post challenge. Viral shedding was determined using reverse-transcriptase real-time PCR. A semi-structured questionnaire and focus group discussions employing participatory mapping and semi-structured interviews were conducted among smallholder goat farmers within three animal health wards in the Mnisi Tribal Authority, a communal farming area within the FMD protection zone with vaccination of Mpumalanga Province.

All the five challenged unvaccinated control goats developed fever within 48 h post challenge with a median fever duration of 5 days. Two unchallenged reduced-dose vaccinated goats maintained as sentinels developed fever at 5 and 9 days in contact, with lesions appearing at 4 and 8 days. Goats presented with nasal discharges and oral mucosal lesions of the lips, and interdigital cleft lesions. The virus caused mild clinical signs and natural transmission to reduced-dose vaccinated in-contact goats occurred. None of the goats vaccinated with the full cattle dose developed secondary FMD lesions. Vaccinated groups had lower temperatures compared to the unvaccinated controls ($P<0.001$). Based on RT-PCR results, goats in the G5 (unvaccinated control) shed more virus compared to all groups except for G4 ($P<0.05$), while goats in the G1 (full dose) shed less

virus than goats in the G4. The results suggest that the G2 (0.67 ml) dose of the vaccine is sufficient to reduce viral shedding after heterologous challenge with a FMDV SAT1 pool. The movement network study identified several FMD high-risk locations within the study area that can be used to prioritise vaccination programmes and targeted disease surveillance. The study further identified communities at high risk of disease occurrence that might play significant roles in disease spread to disease free areas. Four locations in the FMD free zone of the country (Nelspruit, Tzaneen, Barbertone and Leboeng) were identified as having connections with the movement of goats from the study area. Thulamahashe, a nearby town in Mpumalanga Province and Giyani in Limpopo were the two locations with high degree of cattle movement from the study area. Findings from this study further demonstrated that goats are moved without official movement permits to the FMD free zone of the country, with most farmers being unaware of the need to obtain official veterinary movement permits. These animal movements put the country at risk of FMD outbreaks within the free zone.

Information gained from this study contributes to a greater understanding of the role of goats in the epidemiology of FMD in an endemic setting and their response to vaccination. This will provide an opportunity to facilitate FMD endemic countries within the Southern African Development Community (SADC) region and Africa in general to progressively reduce the impact of FMD.

CHAPTER ONE

BACKGROUND AND INTRODUCTION

1.1 Background

Foot-and-mouth disease (FMD) is an important livestock disease that affects all cloven-hoofed domestic (Grubman and Baxt, 2004) and wildlife species (Bengis et al., 2002; Jori and Etter, 2016). FMD control within the South African wildlife interface areas involves routine mass vaccination campaigns for cattle, regular inspection of cattle and other livestock at communal dip tanks, animal movement control through permit systems and the maintenance of veterinary cordon fences between wildlife and susceptible livestock populations (DAFF, 2014). Many farmers within interface areas depend on the livestock sector for their livelihoods and understanding the epidemiology of FMD in cattle and small ruminants is expected to improve livestock production, food security and enhance economic empowerment.

FMD is known to affect all cloven-hoofed livestock but there are relatively few reports of clinical disease in small ruminants (Gibson and Donaldson, 1986; Barnett and Cox, 1999; Elnaker et al., 2013; Elnekave et al., 2016; Hughes et al., 2002). Evidence of viral exposure in small ruminants has been reported to occur with the possibility of transmission to susceptible cattle populations (Balinda et al., 2012; Legesse et al., 2013; Hyera et al., 2006; Parida et al., 2008; Paton et al., 2009; Lazarus et al., 2012; Elnekave et al., 2016; Rout et al., 2014). Furthermore, the UK outbreak in 2001 was predominantly spread by sheep (De la Rua et al., 2001). Goats, as with all other cloven-hoofed species, have been reported to be experimentally infected with FMDV (Alexandersen et al., 2002; Anderson et al., 1976; McVicar and Sutmoller, 1968). However, limited reports of natural infections due to the Southern African Territories (SAT) viruses have been produced for this species. In India, a severe form of FMD has previously been reported in goats that included a

high fatality proportion (Shankar et al., 1998). An FMD carrier state has been documented in goats (Anderson et al., 1976; McVicar and Sutmoller, 1968; McVicar and Sutmoller, 1972). Goats have also been incriminated in the introduction of FMDV into disease free countries through illegal trade (Gleeson et al., 2003; Kitching, 1998; Kitching and Hughes, 2002; Leforban and Gerbier, 2003).

In southern Africa, African buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*) and other wild ungulates are involved in the maintenance and transmission of FMDV to susceptible cattle populations at wildlife interfaces (Bastos et al., 2000; Dyason, 2010; Sutmoller et al., 2000; Hargreaves et al., 2004; Thomson et al., 2003; Vosloo et al., 2002; Vosloo et al., 2009). Smallholder farmers within the FMD protection zone with vaccination of South Africa keep livestock collectively (small ruminants and cattle) and these animals are often housed together or in close proximity. However, small ruminants (sheep and goats) are not currently included in the prophylactic FMD vaccination programme and their role in FMD epidemiology at the interface is currently unknown. Goats are reared mostly by the smallholder farmers within the FMD Protection zone of the Mpumalanga Province in South Africa either due to the arid nature of the area and the adaption of goats for the climate. Sheep are kept within intensive farming systems in the FMD-free zone of the country. Goats might have played a role in a previous FMD outbreak (Dr Bjorn Reininghaus, personal communication) and we have identified seropositive sheep and goats during recent FMD Southern African Territories 2 (SAT2) outbreaks within the former FMD free zone of Limpopo Province (unpublished data).

FMD control in endemic situations requires the induction of protective immunity in a sufficient proportion of the population. This is best achieved by immunisation programmes that elicit long-term protection, which is the hallmark of adaptive immunity. Vaccines are fundamental for the

control of FMD and other important livestock diseases. FMD vaccines are one of the most widely used livestock vaccine with over 2 billion doses administered annually (Knight-Jones et al., 2014).

1.1.1 Goat production in South Africa

Goats were mankind's first domesticated animal that have been farmed in both harsh climates and more modern dairy farms throughout the world (Anonymous, 2019). They are kept by a large part of the population in the rural areas of South Africa (Els, 1996). Goats produce milk, meat, skin, cashmere, mohair and play an important role in religious and cultural ceremonies. South Africa produces approximately 3% of Africa's goats (Anonymous, 2019). Goats are found throughout the country with the Eastern Cape, Limpopo and KwaZulu Natal Provinces being the largest producers (DAFF, 2019), accounting for approximately 70% of the total live goats (Table 1.1). Goats are bred in South Africa mainly for meat, milk and fibre. The most important goat breeds for meat are the Boer goat, Savanna and Kalahari red, which can also produce small quantities of cashmere (Mdladla et al., 2016; Simela and Merkel, 2008). Chevon (adult goat meat) has been described to be 50-60% lower in fats than beef but has the same protein content (Anonymous, 2019). It is a red meat that is a potential competitor to beef and mutton (Simela and Merkel, 2008). Chevon is also lower in fat than chicken (Ivanovic et al., 2016). In 2016, Lesotho was the leading importer of chevon, accounting for 74% of South Africa's export market (Anonymous, 2019). South African farmers produce about 60% of the world's production of mohair, mostly in the Karoo region of the Eastern Cape, with most mohair exported for foreign earnings (Anonymous, 2019).

1.1.2 Indigenous goats in South Africa

The term "indigenous goat" refers to various breeds of goats reared by smallholder farmers that contribute primarily to family needs in the form of meat and to a lesser extent milk, depending on the preference of the community. Some indigenous goats also grow cashmere during winter. The

indigenous goat was historically found in the Eastern Cape Province, but it has now been adopted by more breeders and farmers throughout the country (Anonymous, 2019). The indigenous goat is neither regarded as a commercial meat goat, due to its small carcass, nor is it regarded as a milk producing goat as it only produces enough milk for its kids. It has value in the cultural meat market because it is used for religious and other cultural celebrations in South Africa. Commercial goat farmers mainly raise Boer goats for meat and Angora goats for fibre, while smallholder farmers in communal areas mainly own indigenous goats. Indigenous goats represent about 65% of the goats in South Africa (Anonymous, 2019), but selected breeding of indigenous goats created the three meat goats – the Boer goat, Kalahari Red and Savanna.

Table 1.1 Estimated livestock population in the Republic of South Africa (August 2019).

Province	Cattle	Sheep	Pigs	Goats
Western Cape	498,000	2,633,000	153,000	207,000
Northern Cape	442,000	5,344,000	19,000	470,000
Free State	2,111,000	4,573,000	113,000	216,000
Eastern Cape	3,104,000	6,540,000	85,000	2,019,000
KwaZulu Natal	2,465,000	657,000	145,000	682,000
Mpumalanga	1,261,000	1,567,000	111,000	80,000
Limpopo	910,000	204,000	335,000	909,000
Gauteng	243,000	87,000	156,000	24,000
North West	1,575,000	608,000	281,000	669,000
Total	12,808,000	22,213,000	1,398,000	5,276,000

Source: Department of Agriculture, Forestry and Fisheries: National Livestock Statistics, August 2019.

1.1.3 History of foot-and-mouth disease in South Africa

FMD was first officially reported by Hutcheon in South Africa in the year 1892 when an outbreak

occurred in Griqualand West (Hutcheon, 1892). Although the disease existed in South Africa prior to this time and was considered a scourge among farmers. Two years after the first report, limited epidemics occurred in different parts of the country with low case fatality. No further outbreaks were reported after 1895 until April 1903 when a shipment of cattle from Argentina brought the disease to the Cape Peninsula (Thomson, 1994). However, FMD was confined to two places: a farm where the imported cattle were kept and a local dairy that harbored a runaway heifer from the Argentine shipment. Both premises were immediately placed under strict quarantine and thoroughly disinfected. At the end of July of the same year, there was no evidence of the disease and the quarantine restrictions were lifted.

FMD disappeared in the Southern African Development Community (SADC) region until April 1931, when a SAT2 outbreak was reported in Zimbabwe (Thomson, 1994). The recognition of FMD was a great concern to the authorities at that time because the ability of African buffalo to act as reservoir of infection for the SAT FMDV serotypes was unknown and many people believed that the infection had been introduced by imported animals or animal products. Decades after this outbreak, cases of FMD were regularly reported along South Africa's border with Zimbabwe and Botswana until the 1960s (Thomson, 1994). In South Africa, only the SAT1, SAT2 and SAT3 FMD viruses occurred prior to the introduction of serotype O in September 2000 (in KwaZulu Natal Province) (Dyason, 2010).

1.1.4 FMD vaccination in South Africa

FMD vaccines have been widely applied for the control of disease in the SADC since the mid-1960s (Thomson, 2008). Live attenuated vaccines were initially employed and by the late 1960s these had been replaced with inactivated vaccine preparations, which are still in use within this region. From the late 1970s, the Botswana Vaccine Institute (BVI) produced vaccine using the

Frenkel system (Thomson, 2008), but later switched over to suspension cultures. The BVI has been the major supplier of FMD vaccine in the region and the entirety of Africa. The then Exotic Disease Division of the Onderstepoort Veterinary Institute (OVI) began manufacturing FMD vaccine in the mid-1980s. However, production was suspended in 2006 due to the technical difficulty in adapting the SAT-viruses for growth in suspension culture. The FMD vaccine currently used for the control of disease in the protection zones of the country is an alhydrogel-saponin inactivated trivalent (SAT1, SAT2 and SAT3) preparation produced by the BVI. However, there is an on-going initiative at producing local high-potency vaccine containing representative viruses of the region with the aim of addressing uncertainties surrounding recurrent FMD outbreaks within the FMD Protection zones of the country. Cattle within the protection zone with vaccination (Figure 1.1), are vaccinated against FMD at least three times annually (from January, May and September) and branded with a F on the right side of the neck for identification (Lazarus et al., 2018). Goats and sheep are not currently included in the routine mass vaccine campaigns in South Africa.

1.2 Problem Statement

FMD affects all cloven-hoofed livestock and wildlife species. However, cattle are the primary cloven-hoofed animal included in prophylactic vaccination programmes. Sheep and goats are not currently vaccinated against FMD in South Africa and their role in the epidemiology of the disease and responses to FMD vaccination are currently unknown. This might be due to the common perception that these species are not important in the epidemiology of the disease. Other factors might be the budgetary allocations for the cost of vaccines and logistics associated with the control programme. However, there were times that these species were previously vaccinated during an outbreak within the FMD Protection zone of Mpumalanga Province (Mpumalanga Veterinary Service).

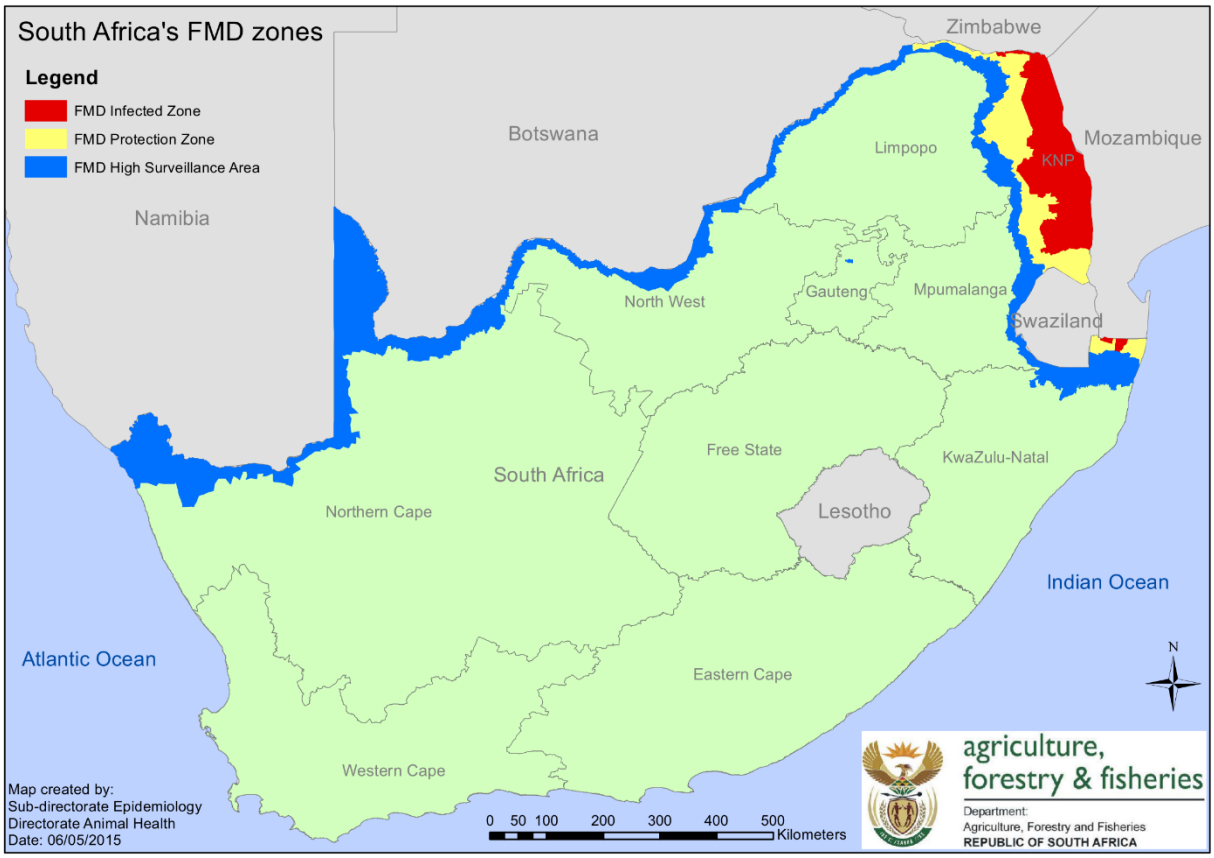


Figure 1.1 Map of South Africa showing the FMD control zones, cattle are only vaccinated against FMD within the FMD protection zone with vaccination. With permission from DAFF: Directorate of Animal Health/Epidemiology 2020.

1.3 Aims and objectives of the study

The aim of this study is to investigate the epidemiology, vaccination and control of FMD within the FMD protection zone with vaccination with emphasis on goats and the Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa. The Mnisi Tribal Authority is a communal farming area adjoining the Kruger National Park and situated within the FMD protection zone of the province. The University of Pretoria, Faculty of Veterinary Science along with the Mpumalanga Tourism and Park Agency has established an Animal Health Community Outreach within the locality making it a suitable hub for research on infectious animal diseases including

FMD.

To accomplish this, the study focused on the following specific objectives:

1. Describe the clinical presentation of FMDV SAT1 infection in experimentally challenged South African indigenous goats. This virus serotype was selected due to its ability to establish clinical disease in goats relative to other serotypes evaluated for the study.
2. Determine the efficacy of a double oil-emulsion inactivated FMD vaccine containing SAT1 virus in goats.
3. Describe the livestock movement network among smallholder farmers within the Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa.

CHAPTER TWO

LITERATURE REVIEW

2.1 The FMDV

Foot-and-mouth disease virus (FMDV) is a small, non-enveloped virus in the genus *Aphthovirus,* within the family *Picornaviridae*. The virus has a positive-sense single stranded RNA genome of approximately 8400 nucleotides in length containing one large open reading frame (ORF) encoding four capsid structural proteins and eight non-structural proteins (Brito et al., 2016). The capsid structural viral protein (VP1), which is coded by the VP1 gene, is known to have important antigenic properties and is the virus protein traditionally used for sequence analysis (Belsham, 2005). The high mutation rate that occurs within the FMDV genome has been attributed to the FMDV RNA-dependent RNA polymerase (3D) lacking proof-reading ability (Domingo and Holland, 1997; Drake and Holland, 1999).

FMDV naturally infects cloven-hoofed species causing an acute illness characterised by fever and lesions of the oral cavity, coronary band, interdigital space and teats in lactating cows (Jamal and Belsham, 2013; Kitching, 2002). It is one of the world's most important animal pathogens, responsible for losses in livestock trade, as well as frequent and highly disruptive large-scale epidemics (Paton et al., 2010). Infection with FMDV elicits a rapid humoral immune response in both vaccinated and non-vaccinated animals. FMDV structural proteins stimulate the production of neutralising antibodies that provide protection against future disease challenge, though antibodies produced against non-structural proteins are not believed to offer clinical protection (Grubman and Baxt, 2004).

Seven antigenically divergent FMDV serotypes have been described, namely serotypes A, O, C (the so-called European types), Asia-1 and the three Southern African Territories (SAT) types 1,

2 and 3. FMDV serotypes A, O, C and Asia-1 constitute a distinct lineage, separate from the SAT viruses (Vosloo et al., 2009). The serological classification is based on the inability of viruses from different serotypes to induce cross protection in animals (Esteban et al., 2003). However, subsequent research findings have also demonstrated antigenic and genetic variations within individual FMDV serotypes (Tosh et al., 2003; Samuel and Knowles, 2001; Samuel et al., 1990; Mateu et al., 1988).

2.2 Characteristics of FMDV

FMDV contains a single-stranded RNA genome of approximately 8400 nucleotides. The capsid has the classical structure of the *Picornaviridae*, consisting of a non-enveloped capsid with icosahedral symmetry, 28-30 nm in diameter, and composed of 60 asymmetrical protomers (Sáiz et al., 2002). Each protomer consists of four structural polypeptides, VP1, VP2, VP3 and VP4. The VP1, VP2 and VP3 are exposed on the surface of the virus, while VP4 is internally located (Jamal and Belsham, 2013). The virion consists of approximately 70% protein and 30% RNA, with a relative molecular mass of 8.5×10^6 daltons (Sobrino et al., 2001). The RNA has three separate parts, which include the 5' untranslated region (5' UTR), a long coding region and a 3' untranslated region (3' UTR) (Jamal and Belsham, 2013).

Picornaviridae are typically stable at pHs between 3 and 9 (Thomson and Bastos, 2004). However, FMDV is different from other members of the *Picornaviridae* due to its lability at a pH below 7 (Mason et al., 2003). The virus can survive in lymph nodes and bone marrow at neutral pH, but it is destroyed in the muscle when the pH drops below 6 (i.e. rigor mortis). The virus capsid becomes unstable and the ribonucleoprotein is dissociated when the virus is exposed to acidification (Van Vlijmen et al., 1998). The acid lability of the virus is responsible for efficient viral uncoating and endocytotic entry into the host cell.

The virus is relatively resistant to the effect of heat with considerable variation among serotypes and strains (Thomson and Bastos, 2004). FMDV survives best in aerosols at cool environmental temperatures and when the relative humidity exceeds 60% (Soren Alexandersen et al., 2002a).

2.3 The epidemiology of FMD

2.3.1 Worldwide distribution of FMDV

FMDV serotypes are not equally distributed in the regions of the world where the disease still occurs. In Africa, six of the seven FMDV serotypes have occurred and the reported distribution of outbreaks by country and serotype since 1948, and between 1990 and 2013 have been recently reviewed (Tekleghiorghis et al., 2016; Vosloo et al., 2002). However, FMD is considered to be underreported in Africa either due to its endemic status or the perceived low mortality in adult animals compared to other diseases. Therefore, the currently available information might not be an accurate representation of the disease situation on the continent. Nevertheless, it is clear that serotypes O, SAT1 and SAT2 are widely distributed while serotypes A and SAT3 have a more restricted distribution (Tekleghiorghis et al., 2016). FMDV serotype C does not appear to currently be in circulation, as it was last reported in Kenya in 2004 (Sangula et al., 2011). FMDV serotype SAT3 has not been reported as the cause of disease in livestock outside southern Africa since it was last reported in buffalo in East Africa during the 1970s (Hedger et al., 1973). FMDV serotype SAT2 has been reported more frequently in domestic livestock compared to SAT1 and SAT3 (Tekleghiorghis et al., 2016; Vosloo et al., 2002).

FMD is still endemic in most parts of Africa, Asia and the Middle East (Figure 2.1). In Latin America, most countries apply zoning and are recognised as FMD-free, either with or without vaccination (Brito et al., 2017; OIE, 2019). Australia, New Zealand, Indonesia, North America and continental Western Europe are currently free of FMD (OIE, 2019). However, FMD is a

transboundary animal disease that can occur sporadically in any typically free area as has recently occurred (January and November, 2019) in South Africa (DAFF 2019a; DAFF 2019b). In recent times, there have been periodic incursions of serotypes SAT1 and SAT2 into the Middle East (Brito et al., 2017; Donaldson, 1999).

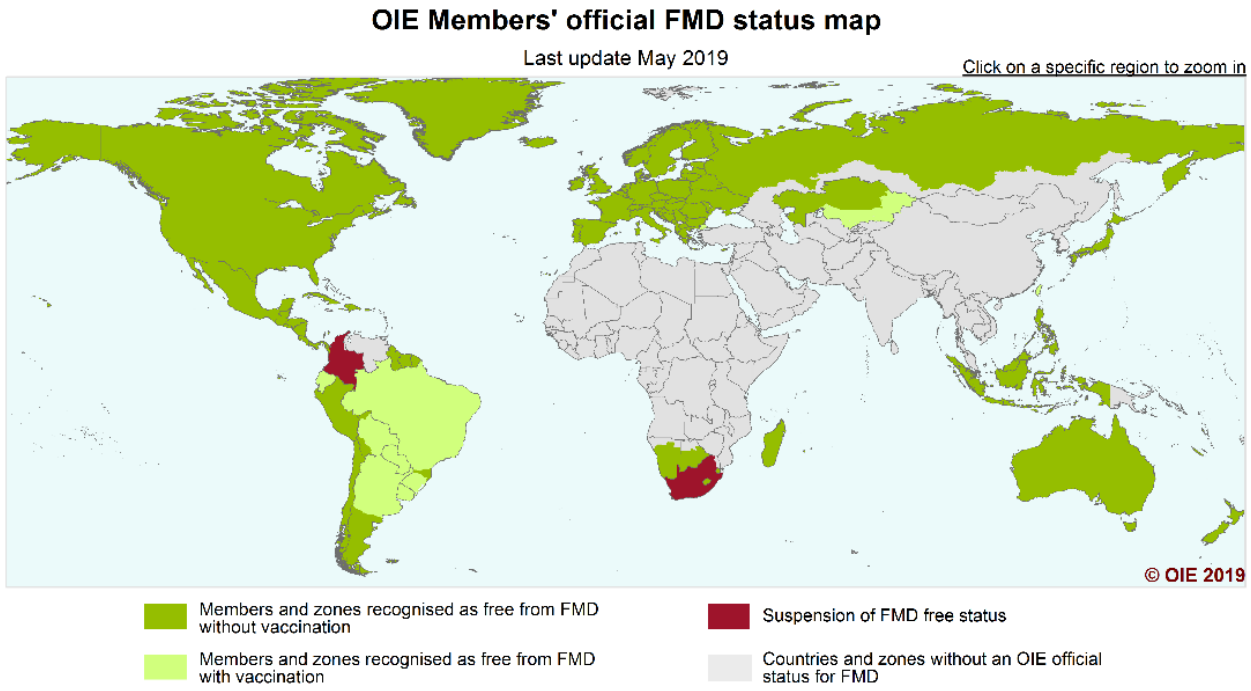


Figure 2.1 OIE Member's official FMD status as at May 2019, adopted from the OIE website, http://www.oie.int/en/animal-health-in-the-world/official-disease-status/fmd/en-fmd-carte/with copyright permission from the OIE, 2019 (Accessed on 23rd July, 2019).

2.3.2 FMD situation in southern Africa

Livestock farming forms an integral part of the rural economy of most Southern African Development Community (SADC) member states. More than 75% of livestock production in this region is within extensive management systems and is therefore susceptible to numerous

challenges including infectious animal diseases such as FMD (Thomson, 2009). Within this region, South Africa, Botswana and Namibia have complied with the World Organisation for Animal Health (OIE) standards and regulations to certify FMD free zones where vaccination is not practised. In South Africa, for the purpose of disease control, the country has been classified into the FMD infected zone (the Kruger National Park and adjoining nature reserves where African buffaloes are found), the FMD protection zone with or without vaccination and the majority of the country that forms the FMD free zone (DAFF, 2014). However, South Africa has recently lost its free zone status due to FMDV SAT2 outbreaks in the previously free zone during 2019 (DAFF 2019a; DAFF 2019b). In southern Africa, cattle are frequently raised within communal farming areas at the wildlife-livestock interface. With the recent establishment of trans-frontier conservation areas (TFCA) across southern Africa, there is the hope to enhance biodiversity, conservation and eco-tourism among rural communities living at this interface (Brito et al., 2016). However, due to husbandry practices and the proximity of the communal farmers to the interface areas, interactions between wildlife and susceptible livestock create a risk for the transmission of infectious diseases. In extensive husbandry systems, disease due to FMDV infection is often mild and of little concern to animal owners, as most infected animals tend to recover within weeks (Thomson and Bastos, 2004). Three serotypes of FMDV, SAT1, 2 and 3 are maintained within African buffalo (*Syncerus caffer*) populations in the SADC region, with serotypes O and A also occurring in cattle in Tanzania (Thomson, 1994). Historical evidence suggests that the SAT viruses evolved in buffalo in sub-Saharan Africa while serotypes A, O, C and Asia-1 might have evolved in livestock (Vosloo et al., 1996; Bastos et al., 2001; Bastos et al., 2003). The evolution of the SAT FMDV in the African buffalo in the SADC has made the region to be endemically infected, this limiting trade prospects.

Most southern African countries have established complicated and capital intensive methods to control FMD (Thomson and Bastos, 2004). However, FMD outbreaks within SADC have increased in frequency, and in most cases, the outbreaks have persisted for longer times (Jori et al., 2016; Penrith and Thomson, 2012). Overall, traditional FMD control measures have become inadequate in some parts of the SADC during the last 10-15 years (Lazarus et al., 2018; Thomson et al., 2013;Vosloo and Thomson, 2016). Several countries in SADC have reported FMD outbreaks during the past decades, with South Africa officially reporting outbreaks within the FMD free zone of the country in February 2011, January 2019 and November 2019 (OIE-WAHID, 2017, 2018; Vosloo and Thomson, 2017; DAFF 2019a; DAFF 2019b). The official World Organisation for Animal Health (OIE) recognised FMD free status without vaccination of South Africa has been suspended after two consecutive outbreaks in the previously FMD free zone of Limpopo Province. Several outbreaks have occurred within the FMD protection zones of Mpumalanga and Limpopo which did not affect the free zone status of the entire country. During the period 2005 – 2016, seven FMD outbreaks occurred, which was a combination of SAT1, SAT2 and SAT3 and affecting a total of 31 communal dip tanks with a total of over 1000 cases in cattle (https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review/viewsummary?fupser=&dothis=&reportid=26739, Accessed on 2020/07/02). Therefore, there is a need for the development of a regional strategy for the progressive management of FMD in sub-Saharan Africa.

2.4 Transmission of FMDV

FMDV is usually spread by the movement of infected animals and contact with contaminated materials. Susceptible cattle are typically infected by the respiratory route, but the virus can also enter through skin abrasions. During acute infection, virus is shed from ruptured vesicles and in bodily excretions and secretions, including breath, milk and semen (Alexandersen et al., 2003;

Paton et al., 2018). Cattle are highly susceptible to infection by the respiratory route (Kitching, 2002). Calves can also be infected with FMDV by the consumption of contaminated milk from infected cows. Among all susceptible livestock species evaluated, cattle and sheep are the most likely to be infected by low viral doses via aerosols generated by other infected animals. Cattle have a large respiratory volume compared to small ruminants and therefore are more likely to be infected by the airborne route (Gibson and Donaldson, 1986). However, pigs are known to excrete the largest quantities of airborne virus (Alexandersen et al., 2002; Alexandersen and Donaldson, 2002), consequently the most likely pattern of airborne FMD spread is from pigs to cattle.

2.5 Clinical signs of FMD in domestic ruminants

2.5.1 Clinical signs of FMD in cattle

FMDV infection in cattle has an incubation period of between 2 and 14 days following natural infection (Kitching, 2002). However, this depends on the viral dose, virus strain and the susceptibility of the host species. The clinical signs of FMD in naïve cattle is usually obvious with early signs of sudden loss of milk production in dairy animals that is often accompanied by anorexia, depression and fever (Donaldson, 2004). This is usually followed by the occurrence of vesicles on the tongue, hard palate, dental pad, lips, gums, muzzle, coronary band, interdigital space and teats of lactating cows (Kitching, 2002). In the mouth, the early lesions of FMD in cattle often appear as blanched areas in the dorsal epithelium of the tongue (Donaldson, 2004). The disease can have a mortality rate of over 90% in young calves before the development of vesicular lesions due to the predilection of the virus to invade and damage cells of the heart muscle (Kitching, 2002). Mortality in adult cattle due to FMDV infection seldom exceeds 5%. Profuse salivation is observed in acutely infected cattle. Infected cattle can also develop nasal discharge that starts as mucoid but can progress to mucopurulent and cover the entire muzzle. Acutely

infected cattle might stamp their feet due to the painful foot lesions. Some animals become recumbent and resist all attempts at raising them. Lactating cows with teat lesions and cows with foot lesions are predisposed to bacterial infections that can cause secondary mastitis and lameness, respectively. Vesicles on the feet can remain intact for up to two to three days before rupturing. Mouth lesions heal rapidly, with the erosion being filled with fibrin and by day 11 after vesicle formation, they appear as areas of pink fibrinous tissue without normal tongue papillae (Kitching, 2002). However, the healing of foot lesions is often protracted especially when affected by secondary bacterial infections.

2.5.2 Clinical signs of FMD in sheep

The clinical presentation of FMD in sheep is well documented (Kitching and Hughes, 2016; Arzt et al., 2011; Donaldson, 2004; McVicar and Sutmoller, 1972; Littlejohn, 1970) and subclinical infections occur more frequently compared to cattle and swine (Arzt et al., 2011; Gibson and Donaldson, 1986; Cardassis et al., 1966). The incubation period following natural FMDV infection in sheep is between 3 and 8 days (Arzt et al., 2011; Kitching and Hughes, 2002). However, this can be as short as 24 hours following experimental inoculation, or longer up to 12 days. The incubation period depends on the susceptibility of the sheep, virus strain, dose of FMDV and the route of infection. Clinical signs might appear up to 3 days after the start of viraemia, with vesicular lesions failing to develop in a number of infected sheep. Vesicles are less common in the mouth compared to cattle (Kitching and Hughes, 2002; Barnett and Cox,1999). Lameness is usually the first indication of FMD in sheep even though the infected animal might develop fever and separate itself from the rest of the flock (Kitching and Hughes, 2002). Mortality rates can be high in young animals with typical myocardial lesions found at necropsy (Musser, 2004; Kitching and Hughes, 2002). FMDV can persist in sheep beyond the resolution of clinical disease (Burrows, 1968).

However, the duration of persistence in sheep has been reported to be shorter than in cattle, which has been reported to be over three years in some instances (Kitching, 2002), with the virus being isolated in sheep up to 9 months after experimental infection (McVicar and Sutmoller, 1968).

2.5.3 Clinical signs of FMD in goats

Goats are highly susceptible to FMDV infection by the respiratory route, although they have been reported to excrete less aerosolised virus than cattle and pigs (Alexandersen et al., 2003b). FMDV has been isolated from cattle and goats during outbreaks in Africa (Legesse et al., 2013). Thus, including goats in routine vaccinations during outbreaks within endemic settings might limit the risk of subclinical disease in this species and promote disease control. There is relatively little published literature on FMD in small ruminants, with most of the studies focusing on sheep rather than goats. Available literature, suggests that the pathogenesis is similar in both species to what is seen in cattle except for some distinct pre-viraemic, viraemic and post-viraemic phases as it applies to each species (Arzt et al., 2011). The severity of the disease in goats is also dependent on the strain of virus, breed of animal as well as the environmental conditions (Donaldson, 2004). Both sheep and goats inoculated with serotype O_1 Manisa FMDV exhibited clinical signs of the disease including inappetence, panting, pyrexia (≥40°C), lameness and vesicles on the feet and in the mouth at 2 to 5 days post-challenge (Madhanmohan et al., 2010). This study documented the isolation of FMDV from the blood and nasal secretions of goats. Viraemia was detected in these animals at 2 to 5 days post-challenge. Viral RNA was detectable in the blood of animals between 2 to 10 days post-challenge, and in the nasal secretions of animals at 3 to 35 days post-challenge. All animals excreted virus in the oropharyngeal fluid up to 35 days post-challenge. The animals were also reported to be positive for antibodies against non-structural proteins from 15 to 35 days post-challenge. The clinical signs of FMDV serotype O infection has been described in an isolated

flock of Saanen goats (Mavridis, 2018). The author reported that the disease first affected kids and older goats later presented with clinical signs in the following 5 to 6 days. Affected animals presented with high fever (up to 41.9°C), diarrhoea, frothy nasal discharges, excessive salivation, lacrimation, small vesicles and necrosis of the mucosal membranes of the oral cavity. In another study, two goats infected with O/JPN/2010 showed clinical signs, high levels and long-term excretion of virus and efficient virus spread by direct contact (Onozato et al., 2014). The affected goats in this study had small vesicles in the interdigital area, on the bulbs of the heels, and around the coronary bands of the feet at 3-4 days post-challenge. Small vesicular lesions appeared in the interdigital areas, on the bulbs of the heels, and around the coronary bands at 7-8 days post-challenge in two in-contact goats. Excessive salivation and depression were observed in one of the in-contact goats 6-8 days post-challenge. The same goat presented with vesicular lesions on the tongue, lips and the dental pad beginning at 6 days post-challenge (Onozato et al., 2014). Some strains of FMDV have been reported to cause severe lesions in goats whereas infection with others might only cause mild clinical disease. Extra care is therefore necessary during individual examinations and a high proportion of animals might need to be examined in a herd to detect the disease. Indigenous goats in East and Southern Africa have been reported to suffer inapparent FMD infection (Thomson, 1994). Experimental direct contact studies using the intra-nasal route for inoculation have estimated an incubation period of 2-8 days in goats (Kitching and Hughes, 2002; McVicar and Sutmoller, 1972). However, the incubation period for FMD is highly variable, and this could depend on the strain and dose of virus, the route of transmission, the animal species in addition to management conditions (Alexandersen et al., 2003). As in cattle and pigs, fever and vesicles are the hallmarks of clinical FMD in goats and this typically occurs 12-48 h after the onset of viraemia (Arzt et al., 2011). The first sign of FMD in a herd of goats is often a high incidence

of lameness accompanied by depression and anorexia. Pyrexia and the sudden death of young stock can also occur (Donaldson, 2004). The cause of death in young stock is typically heart failure due to multifocal necrosis of the heart muscle. In the early stage of the disease, especially in milking goats, a sudden drop in milk production occurs. Vesicles might be present on the teats and vulva. Oral lesions have been reported to occur more commonly in goats relative to sheep with some strains of FMDV (Olah et al., 1976). Although in a field outbreak affecting both sheep and goats with the same virus, clinical signs were reported to be milder in affected goats (Arzt et al., 2011a). However, there are also field reports of severe clinical signs occurring within goats (Shukla et al., 1974). Overall, both viral and host factors likely contribute to the variability in clinical signs of the disease. Early lesions in the mouth of goats are reported to be small blanched areas of necrotic epithelium, most often on the dental pad. Fluid filled vesicles are an unusual occurrence and if they do occur, they are transient. Erosions/ulcers might also occur on the gums, inside the lips and occasionally on the tongue. The tongue erosions/ulcers generally occur as multiple small (0.5 to 1.0 cm) areas on the dorsum (Donaldson, 2004). Viral persistence occurs in goats with a relatively lower prevalence compared to sheep (Madhanmohan et al., 2012; McVicar and Sutmoller, 1972).

2.6 Global control of FMD and the Progressive Control Pathway (PCP)

Following the successful global declaration of rinderpest eradication by the OIE in 2011, there was a renewed effort toward a global control and eradication of FMD and other transboundary animal diseases. The Progressive Control Pathway for Foot and Mouth Disease (PCP-FMD) was developed by the United Nations-Food and Agriculture Organisation (FAO) and the World Organization for Animal Health (OIE) to facilitate FMD endemic countries to progressively reduce the negative impacts of FMD. The PCP-FMD has been adopted as a working tool by the FAO and

OIE in the design of national and regional FMD control programmes (FAO, 2011, 2018). Within this strategy, countries that are usually free of FMD but detect an incursion could not join the pathway. Instead they would be required to eradicate the disease and reapply directly to the OIE for re-instatement of an officially recognised FMD-free status. The PCP-FMD is a set of control activities combined in stages (Figure 2.2), that when fully implemented should enable countries to apply for OIE-endorsement of a national control programme using vaccination or official freedom from FMD with or without vaccination.

The PCP-FMD consist of two distinct domains: (i) a Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs) pathway from 0 up to and including stage 3 and (ii) an OIE pathway beyond stage 3 (FAO, 2018).

Identification of risk factors such as circulating virus serotypes, livestock movement patterns and FMD high-risk locations in disease endemic settings forms the basis for the progressive control pathway. Several studies have highlighted the role of livestock movement in the spread of FMD through pastoralism and trade across sub-Saharan Africa (Di Nardo et al., 2011; Tekleghiorghis et al., 2016). In Nigeria, cattle markets have been reported to play an important role in the epidemiology of FMD (Ehizibolo et al., 2019, 2017; Fasina et al., 2013).

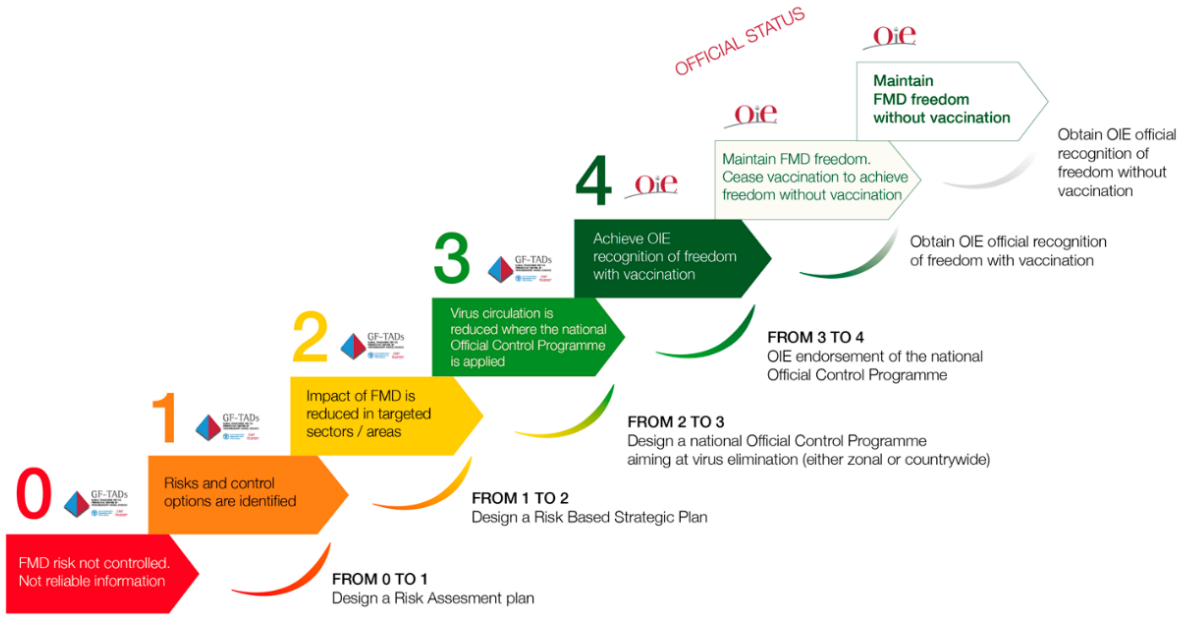


Figure 2.2 The PCP-FMD consist of two distinct domains: (i) a Global Framework for the Progressive Control of Transboundary Animal Disease (GF-TADs) pathway from Stage 0 up to and including stage 3 and (ii) an OIE pathway beyond Stage 3. Stage progression in the Progressive Control Pathway, adopted from the Progressive Control Pathway for FMD control (PCP-FMD), Principles, Stage Descriptions and Standards, EuFMD, OIE, FAO, 2018. With Permission from the FAO, 2019.

2.7 Control of FMD in South Africa

In South Africa, FMD control measures include the separation of wildlife from susceptible livestock populations using electrified (or high impact non-electrified) fences, clinical surveillance of susceptible livestock, routine vaccination of cattle and movement control of susceptible livestock, wildlife and their products. The specific control measures implemented depend upon the location within the country.

In the FMD infected zones of the country, the keeping of livestock is strongly restricted. If cattle are kept within the infected zones, then they should be identified using official (ZAF) green ear-

tags. Inspection of cattle must be performed every 7 days and inspection of small stock (i.e. goats, sheep and pigs) performed every 28 days. Routine mouth examinations must be performed and recorded on at least 10 cattle, randomly selected from the presented cattle on each inspection day and at every inspection point. Susceptible game species, especially impala, must also be inspected.

In areas that fall within the protection zone with vaccination, cattle are inspected at designated dip tanks every 7 days with small stock inspected every 28 days. All cattle in the protection zones with vaccination must be identified using official (ZAF) green ear-tags. Upon suspicion of FMD, serological and virological surveillance must be instituted in accordance with the current FMD Veterinary Procedural Notice (DAFF, 2014).

All cattle (irrespective of age) should be vaccinated using a standard potency (3PD50) vaccine containing serotypes SAT1, SAT2 and SAT3 every 4 months against FMD according to the vaccine directions, which includes revaccination of first time vaccinated cattle after 3-4 weeks (DAFF, 2014). During vaccination campaigns, the vaccination dates, herd identities and number of cattle vaccinated are recorded in the cattle registers by authorised Animal Health Technicians. Movement of cattle is only allowed if the animals originate from a herd that has evidence of being previously vaccinated. A permanent letter “F” is branded on the right side of the neck of the cattle when first vaccinated for FMD. Disease control fences that prevent contact between potentially infected wildlife and susceptible animals are maintained and regularly inspected by veterinary officials (Jori et al., 2011).

2.8 FMD vaccine

Vaccines are fundamental to the successful control of FMD and other transboundary animal diseases. FMD vaccines are the most widely used livestock vaccine with over 2 billion doses produced annually (Knight-Jones and Rushton, 2013). FMD vaccines are biological formulations

containing one or more chemically inactivated cell-culture derived seed virus strain preparations blended with a suitable adjuvant and excipient (OIE, 2018). Conventional FMD vaccines are formulated as either aqueous or oil-based preparations and presented as either a monovalent or multivalent formulation depending on the epidemiological situation. An aqueous vaccine, which is mostly used in ruminants, is prepared by adsorbing the virus on to aluminum hydroxide gel and saponin (OIE, 2018). Oil-adjuvant vaccines are usually formulate using mineral oils (Barnett and Carabin, 2002; Cloete et al., 2008; Doel, 2003). Most of the currently applied FMD vaccines are produced from virus generated in cell culture suspension, inactivated using binary ethyleneimine and formulated with either an aluminum hydroxide saponin or an oil-based adjuvant (Rodriguez and Gay, 2011; Doel, 2003; Barteling and Vreeswijk, 1991). FMD vaccine production requires highly biosecure containment facilities to mitigate the risk of viral escape. Another point of concern is the ability to maintain good thermo-stability of a formulated vaccine through the appropriate cold-chain system from the point of production to the field (OIE, 2018; Doel, 2003). While it is a common practice to formulate FMD vaccines containing multiple virus strains in a single vaccine preparation, protection from all included strains cannot be guaranteed due to antigenic and genetic diversity (Doel, 2005). Currently applied FMD vaccines are also limited by the fact that protection from a primary course of vaccination usually only lasts for 4-6 months (dependent on the vaccine potency) necessitating frequent revaccination in endemic settings (Lazarus et al., 2018; Parida, 2009; Doel, 2005). A further limitation of many currently applied FMD vaccines is the inability to differentiate previously infected and recovered animals from vaccinated animals (Robinson et al., 2016). Differentiation of FMDV previously infected from vaccinated animals usually relies on the detection of serum antibodies against the viral non-structural proteins (NSP), which are generated during active viral infection but not after

vaccination with a purified vaccine. However, purified vaccine preparations are sometimes contaminated with traces of NSP and repeated exposure to vaccination can therefore cause the production of anti-NSP antibodies (Sammin et al., 2007; Brocchi et al., 2006; Paton et al., 2006; Robiolo et al., 2006). FMD vaccines can be classified as either a “standard” or a “high” potency vaccine based on their 50% protective dose (PD_{50}) value. Standard vaccines are formulated to contain sufficient antigen to ensure the minimum potency level requirement of at least 3 PD_{50}, while high potency vaccines (>6 PD_{50}) are formulated with an increased amount of antigen to provide a more rapid onset of immunity and a wider spectrum of immunity against closely related field viruses (OIE, 2018).

Several novel FMD vaccines that do not use inactivated antigens have been developed (Diaz-San Segundo et al., 2017); however, their application is limited. These vaccines include an inactivated whole virus marker vaccine, which grows well *in vitro* but are harmless to animals. FMDV lacking the leader protease coding region (leaderless) can be attenuated *in vivo* and used after chemical activation as an inactivated antigen (Chinsangaram et al., 1998; Mason et al., 1997). FMD subunit vaccine candidates have been developed to address the perceived shortcomings of inactivated vaccines, which includes their inability to induce broadly reactive long-term protection, requirements for multiple revaccination and short shelf-life (Diaz-San Segundo et al., 2017). These vaccine candidates include peptide vaccines produced using VP1 isolated from purified virus or produced in *E. coli* (Shao et al., 2011; Wang et al., 2002; Zhang et al., 2015). DNA vaccines are also subunit vaccine candidates that represents a promising alternative to inactivated vaccines. The benefits of these vaccines is that they do not require high containment facilities for production, have a relatively stable shelf-life, allow for rapid incorporation of emerging field strain sequences, can incorporate marker genes and can co-express multiple antigenic sites from different serotypes

(Diaz-San Segundo et al., 2017). Early attempts at utilizing a DNA inoculation based strategy designed to produce empty capsids in the organism of the inoculated animals have been described (Cedillo-Barrón et al., 2001; Chinsangaram et al., 1998). Similar studies using cDNA encoding the entire viral genome with a mutation at the cell binding site have also been performed (Ward et al., 1997). However, large quantity of DNA and at least two or three inoculations were required and this only induced a low FMDV-specific neutralizing antibody response and inconsistent levels of protection (Cedillo-Barrón et al., 2001). Several empty capsids vaccine candidates, often called virus-like particles (VLP), have been produced in bacterial or insect cells. These are virus particles lacking the nucleic acids that are produced *in vitro* but are as immunogenic as natural virions (Rowlands et al., 1975). In recent years, interest in the use of the VLPs in cattle has increased (Bhat et al., 2013; Mohana Subramanian et al., 2012; Porta et al., 2013). Chimeric vaccine viruses have also been developed. An intra-serotype SAT2 chimeric vaccine has been developed that elicits a strong neutralizing antibody response against a homologous challenge in host animals (Maree et al., 2015). A chimeric FMDV A24 serotype virus (A24LBRV3DYR) has been constructed in which the Leader protease (L^{pro}) coding region has been replaced with the analogous segment from bovine rhinitis B virus (BRVB) (Hollister et al., 2008). *In vivo* studies showed that the A24LBRV3DYR virus was attenuated in cattle and the chimeric virus also exhibited a low level of virulence in pigs exposed via direct contact (Uddowla et al., 2013). The chimeric virus induced protective immunity in cattle against challenge with A24 wild type virus.

FMD vaccines are typically developed for use in cattle (Doel, 1996). However, FMD vaccines containing the SAT strains have been evaluated for use in cattle and small stock (Maree et al., 2015; Cloete et al., 2008; Hunter, 1996, 1998). Sheep and goats vaccinated with a trivalent (SAT1, SAT2 and SAT3) oil adjuvant vaccine maintained humoral antibody levels >1.6$\log_{10}$ titres for up

to 240 days for all three SAT antigens (Hunter, 1996). Cattle vaccinated with a trivalent (SAT1, SAT2 and A) vaccine were protected against a homologous intra-dermolingual challenge at 21 days post-vaccination (Preston et al., 1982). An intra-serotype SAT2 chimeric FMD vaccine induced strong neutralising antibody titres that correlated with protection against intra-dermolingual FMDV challenge in cattle (Maree et al., 2015). A SAT2 oil-adjuvant vaccine derived from either the wild or thermo-stable SAT2 antigen induced full protection in all vaccinated cattle following a homologous intra-dermolingual challenge 5 months post-vaccination (Scott et al., 2017). An inactivated FMD vaccine was 80% effective for prevention of clinical disease after a homologous SAT2 virus challenge in pigs and no viral shedding was detected (Mouton et al., 2018). In a similar study, high virus-neutralising antibodies were induced two weeks post-vaccination in cattle and a clear immune response was induced after a second vaccination in pigs (Jo et al., 2019). A high potency O_1 Manisa vaccine provided clinical protection following a homologous challenge in goats (Madhanmohan et al., 2012). The same authors reported that one-half of the cattle dose of an oil-adjuvant vaccine was sufficient to induce protective immune responses in goats (Madhanmohan et al., 2011; Madhanmohan et al., 2010). Other successful vaccination studies using FMDV serotype O, A, C and Asia-1 have also been reported in goats (Park et al., 2014; Madhanmohan et al., 2009; Patil et al., 2002).

2.9 Host immune responses

The host immune system consists of a complex interacting network of biochemical and cellular reactions. The entry of pathogens or vaccine into the animal body can alter the expression of a very large number of the host biochemical and cellular molecules. These networks possess redundancies and multiple simultaneous mechanisms working together to ensure the destruction of invading pathogens. This of course, maximises their efficiency and minimises the chances of

individual pathogen successfully evading the host defence mechanisms. The host immune system can be divided into the innate and adaptive immune systems. Immunity to disease pathogens can also be classified as either passive or active. The innate immune system involves non-specific defence mechanisms that include biological barriers and chemical defences such as the skin, mucus membranes and stomach acids, which are activated at the instance of an invading pathogen. Antigen-specific immune responses are a component of the more complex adaptive immune response; the antigen must be processed and recognised before the system creates numerous specific immune cells designed to mount an attack on the pathogen. The adaptive immune mechanisms create a "memory" for future responses towards a specific antigen (Palm and Medzhitov, 2009). However, both arms of the immune system are required for an effective immune response against infections.

2.9.1 Passive immunity

Passive immunity is acquired by the transfer of antibodies produced by one animal to another. Specific antibodies alone have been shown to be highly effective in preventing many viral diseases. This provides protection against some infections, but protection is only for a relatively short time period. Antibody levels will wane during a period of weeks to months and the recipient will no longer be sufficiently protected. Passive immunity can be initiated by either artificial passive immunisation or natural passive immunisation. Artificial passive immunisation involves the administration of antibodies, usually by injection. This has been known to provide temporary protection against infection with the viruses that cause canine distemper, feline panleukopenia and porcine reproductive and respiratory syndrome (PRRS) (Tizard, 2013). Natural passive immunity involves the transfer of maternal antibodies from dam to the foetus or neonate. This protects the neonate for the first few months of life against pathogens in which the dam has had previous

exposure. Natural passive immunity is important for two reasons: 1) it is essential for the protection of the young animal during the first weeks or months of life against pathogens that are present in the environment and 2) maternally-derived antibodies can interfere with active immunisation of the neonate and must therefore be taken into consideration when designing vaccination schedules (Maclachlan and Dubori, 2011). The most common source of passive immunity in domestic animals is the maternal colostrum consumed by neonates.

2.9.2 Active immunity

Active immunity involves the stimulation of the host immune system to produce antigen-specific humoral (antibody) and cellular immune responses. Unlike passive immunity, which is short-lived, active immunity can last for many years, for a lifetime in some circumstances. One way to acquire active immunity is to survive natural infection with the specific disease-pathogen. This will persist and might confer life-long immunity to the disease. Another way to produce active immunity is by vaccination. Vaccines interact with the host immune system and often produce an immune response similar to that produced by natural infection. Vaccination might also induce immunologic memory consistent to what is acquired via natural exposure and disease.

2.9.2.1 Innate immune response

The mammalian innate immune system is a collection of distinct subsystems of cells that work through diverse biological mechanisms. They rapidly respond to invading pathogens by cellular and chemical reactions to block the invading pathogens and minimise tissue damage. Innate immunity is not agent specific and is the first line of defence against invading pathogens. Potential pathogens that invade the epithelial barriers of the skin or mucosa are detected and eliminated by specialised phagocytic cells within the submucosa (Murphy et al., 2008). This arm of the immune response relies generally on the fact that pathogens such as bacteria and viruses differ structurally

and chemically from normal host tissues. Animals make molecules that can kill invading pathogens directly or promote their destruction by phagocytic cells. The innate immune response usually precedes the adaptive immune response, with an interplay between the two pathways enhancing the overall effectiveness (Guzman et al., 2012). Several activities mediate the innate immune response and these include: a) epithelial barriers, b) antimicrobial serum proteins including complement, c) antibodies produced by B lymphocytes, d) activities of phagocytic cells including eosinophils, macrophages and dendritic cells, e) natural killer (NK) cells that can lyse virus-infected cells, f) various cell types including mast cells at the site of viral invasion that produce protective molecules including interferon (INF), g) apoptosis, a process of programmed cell death that can eliminate virus-infected cells and h) small RNA molecules that interfere with virus replication (Maclachlan and Dubori, 2011). Macrophages recognise common features that are present on pathogens through pattern recognising surface receptors (PRRs). Macrophages destroy pathogens through phagocytosis and produce signalling molecules in the form of inflammatory cytokines that activate and recruit other effector cells of the immune system (Murphy et al., 2008). Macrophages are also involved in "mopping up" debris from damaged cells and inactivated pathogens.

2.9.2.2 Adaptive immune response

The adaptive immune response is a component of the overall immune system that is composed of highly specialised systemic cells and processes that eliminate pathogens upon invasion of the host. This response includes antibody (humoral) and cellular components. The humoral component is mediated principally by B-lymphocytes, whereas the cellular immunity is mediated by T-lymphocytes. In addition, dendritic cells, macrophages, natural killer cells and cytokines are all essential components of the immune response. The cytokines that are essential for the adaptive

immune response are principally produced by B- and T-lymphocytes in response to antigen recognition. These cells promote the proliferation, differentiation and activation of other lymphocytes (Maclachlan and Dubori, 2011). The response is antigen specific, taking longer to develop relative to the innate response, and is mediated by lymphocytes that possess surface receptors specific for the epitopes of each pathogen. Adaptive immune responses stimulate long-term memory after infection, meaning that protective immune responses can be quickly reactivated on re-exposure to a previously encountered pathogen.

2.9.2.2.1 Humoral immunity

The humoral immune (antibody-mediated) response involves B cells, which are produced in bone marrow precursors but develop and mature in the Peyers patches. The role of B lymphocytes is to recognise antigens present in the lymphoid tissue or blood. Firstly, antigens bind to B cells and then interleukins or helper T-cells co-stimulate the B cells to be activated. In most cases, both an antigen and a co-stimulator are required to activate B cells and initiate B cell proliferation. The B cells proliferate upon stimulation and produce plasma cells. These plasma cells produce antibodies with the identical antigen specificity as the antigen receptors of the activated B cells and released antibodies then circulate through the body. The B cells also produce memory cells, which provide future immunity against exposure to the same antigen.

2.9.2.2.2 Cellular immunity

The adaptive cellular immune response is primarily mediated by T-cells, which are produced in the bone marrow precursors and travel through the blood stream to the thymus gland where they develop and mature (Tizard, 2013). Two forms of T-cells exist: T helper cells and T killer cells. T helper cells activate the immune system but do not destroy infected cells or pathogens. The major roles of T helper cells are to stimulate B cells to secret antibodies, activate phagocytes, activate T

killer cells, and to enhance the activity of natural killer (NK) cells (Tizard, 2013). T killer cells are cytotoxic, and they recognise and destroy virus infected cells. These cells also play a role in the defence against intracellular bacteria and certain types of cancers. Cellular immunity in response to an antigen does not involve antibody production but relates to the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes and the release of various cytokines where the T-helper lymphocytes (Th/$CD4^+$) and T-cytotoxic lymphocytes (Tc/$CD8^+$) are considered to be the most important. The immune responses involving T cells are pathogen-specific and are controlled by the major histocompatibility complex (MHC) class I and II molecules (Germain, 1994).

2.10 Immunity induced by FMDV and vaccine

2.10.1 Immune responses secondary to FMDV infection

Infection with FMDV has been known to elicit a rapid, strong and lasting antibody-specific immune response in cattle, sheep and pigs (McCullough et al. 1992a; McCullough et al. 1992b; Pay and Hingley, 1987). Together with the early detection of circulating antibodies, there is a rapid clearance of virus from the circulation and a gradual reduction in viral shedding. Following infection with FMDV, IgM has been reported to be the first serum-neutralising antibody to appear within 3-4 days post-infection, and this has been demonstrated to peak in concentration approximately 10-14 days post-infection (Golde et al., 2008; Collen, 1994). Neutralising antibody IgG has been detected 4-7 days post-infection in cattle and becomes the major neutralising antibody by 2 weeks post immunisation (Francis and Black, 1983). Antibodies to natural infection with FMDV has been described to be long-lasting relative to vaccine induced antibodies (Doel, 2005). With respect to intracellular pathogens, it is acceptable that immune responses that depend on the activities of the phagocytes and T-cells, rather than antibody-dependent responses alone have greatest relevance (Pollock and Welsh, 2002). Several subpopulations of T-cells, including

CD4, CD8 and γδ T-cells have been described to interact with infected cells (Denkers and Gazzinelli, 1998; Schaible et al., 1998; Doherty et al., 1996). T-cells derive their specificity through αβ T-cell receptors have fundamental roles in cellular immune responses through their capacity to recognise antigen peptides presented on MHC-encoded molecules (Pollock and Welsh, 2002). $CD4^+$, MHC-II restricted T-cells have a primary role in the production of macrophage activating cytokines including IFN-γ (Pieters, 2000; Boehm et al., 1997). These mediators enhance the ability of infected cells and professional phagocytes to kill or inhibit the activity of the infecting pathogens. $CD8^+$, MHC-I restricted T-cells have a crucial cytotoxic role with the ability to lyse virus infected cells (Stenger, 2001; Milon and Louis, 1993). Type I and type II IFNs are also key components of the early stage immune response to viral infections (Eschbaumer et al., 2016; Reid and Charleston, 2014). All mammalian cells are susceptible to type I IFNs, while the type II IFN receptor is mostly restricted to the gastrointestinal tract and the epithelial cells of the respiratory tract (Sommereyns et al., 2008). FMDV has been described to induce lymphopenia and suppression of the immune response in pigs, characterised by the loss of circulating T cells (Diaz-San Segundo et al., 2006; Bautista et al., 2003). In cattle, a significant level of lymphopenia has been described during the acute phase of FMDV infection (Eschbaumer et al., 2016). However, other studies have demonstrated no changes in the total circulating leucocytes or subpopulations of lymphocytes (Windsor et al., 2011; Juleff et al., 2009). It has also been demonstrated that the T-cell response to mitogen and non-FMD antigens was not affected during the acute stage of FMDV infection, but T-cell FMD-specific responses were detected (Windsor et al., 2011).

2.10.2 Immune response to FMD vaccination

Several previous studies have demonstrated that humoral immune responses to FMDV correlates with protection against challenge in cattle (McCullough et al. 1992a; McCullough et al. 1992b).

Following FMDV vaccination, an animal will produce high levels of neutralising antibodies against the FMDV structural proteins (Pay and Hingley, 1987; Salt, 1993). The first neutralising antibody response is IgM, which is detected in serum 3 to 7 days after vaccination, peaking between 5 and 14 days and a slow decline to an undetectable level by 56 days post-infection (Doel, 1996; Golde et al., 2008; Juleff et al., 2009). Rapid isotype switching causes IgG1 and IgG2 antibodies to be detected from 4 days post exposure, which reaches a peak at 14 to 20 days (Collen, 1994; Doel, 2005; Juleff et al., 2009; Salt et al., 1996). Pigs and cattle that are protected against FMD following vaccination produce higher levels of IgG1 compared to IgG2 (Barnett et al., 2002; Capozzo et al., 2011; Juleff et al., 2009). *In vivo* antibody virus neutralisation is complex and includes the interaction of antibodies with cells and molecules of the innate immune system. FMDV vaccination causes a rapid humoral immune response that can provide protection against re-infection with a homologous FMDV (McCullough et al., 1992b; Pay and Hingley, 1987; Salt, 1993). However, considering the importance of the T cell response during FMDV vaccination, new approaches towards predicting protection following immunization are beginning to incorporate markers of T cell immunity. For example, the combination of virus neutralization test (VNT) data and measures of the level of IFN-γ produced by CD4-expressing T cells in whole-blood re-stimulation allowed for better prediction of clinical protection following vaccination (Oh et al., 2012). Interestingly, the extent of the IFN-γ response to the vaccines tested in the study was not dose dependent and did not always correlate with the VNT titres of the animal. This further raises the question as to which other host-intrinsic factors are involved in determining the magnitude of the IFN-γ producing T cell response to vaccination, and how the response might be augmented by rational improvements to vaccines (Robinson et al., 2016b). The ability to predict cross-protection between vaccine strains and field isolates is an important research objective.

Conventionally, the likelihood of cross-protection has been estimated using virus neutralization tests, liquid-phase blocking ELISA and complement fixation assays, but these techniques are laborious with variable accuracy. In a recent study, the suitability of high throughput avidity and IgG-subtype ELISAs to predict cross-protection in cattle from serum samples was assessed. In cattle immunized against FMDV A24 Cruzeiro and challenged with A/Arg/01, protection against the heterologous strain was associated with higher levels of avidity antibodies to A/Arg/01. Some animals were also observed to have low or undetectable VNT titres, yet they were protected upon challenge, and this was linked to a higher IgG1/IgG2 ratio than non-protected animals (Lavoria et al., 2012). The challenges of identifying correlates of protection following vaccination often reflect a more fundamental lack of knowledge of the events immediately following vaccine administration (Robinson et al., 2016a). In the case of an adenovirus-based FMDV vaccine, some progress has been made by the development of recombinant adenovirus 5 (rAd5) carrying either a luciferase- or GFP- encoding gene in place of FMDV sequences. Inoculation of the traceable rAd5-luciferase particles into cattle documented an association of transgene products with antigen-presenting cells within the inflammatory filtrates, and from 6 hours post-inoculation within the inter-follicular areas of the draining lymph node (Montiel et al., 2013). A further experiment using rAd5-GFP revealed that the addition of an oil-based adjuvant increased the frequency of expression of the fluorescent protein within lymph migratory dendritic cells (DC). In another experiment, the addition of the same adjuvant caused an increase of FMDV-specific IFN-γ and tumour necrosis factor alpha (TNF-α) secreting memory T cells compared to vaccination with the rAd5-FMDV vaccine alone (Cubillos-Zapata et al., 2011).

2.11 Laboratory diagnosis of FMDV

Laboratory diagnosis of FMDV include a variety of direct detection methods including virus

isolation and the demonstration of FMDV antigen or nucleic acid in clinical specimens. The detection of FMDV-specific antibodies can also be used for diagnosis with antibodies to viral non-structural proteins (NSPs) being good indicators of infection, irrespective of the vaccination status of animals. FMDV NSPs are highly conserved proteins and are therefore not serotype specific.

2.11.1 Diagnostic methods for identification of viral agents in clinical specimen

2.11.1.1Virus isolation

Virus isolation is the "gold-standard" diagnostic test employed for the propagation of "live" FMDV and other viruses from clinical specimens (OIE, 2018). The test involves the propagation of live viruses in highly sensitive *in vitro* cell culture systems. FMDV grows in a wide range of primary and continuous *in vitro* cell culture systems. Primary cells are usually processed directly from animal tissues with no passage and can contain a mixture of cell types. Continuous cells are purified established cell lines developed for laboratory use. Primary bovine thyroid (BTY) cells (House and House, 1989) are the most sensitive cell line for the isolation of FMDV from clinical specimens. However, primary pig, calf or lamb kidney cells could serve as alternatives to BTY cells for the primary isolation of FMDV from clinical specimens (OIE, 2018). Continuous cell lines including baby hamster kidney (BHK) cells, lamb kidney cells and pig cell lines IB-RS-2 and MVPK-1 are also susceptible to FMDV infection (Ludi et al., 2017). The foetal goat tongue cell line (ZZ-R 127) has also been shown to be highly sensitive to both wild-type and cell-adapted strains of FMDV (Brehm et al., 2009). This cell line is a rapid and convenient medium for the isolation of FMDV from clinical specimens, which has served as a useful alternative to BTY. Virus isolation assays have proven to be useful for the isolation of FMDV from a variety of clinical specimens from both experimentally inoculated and naturally infected animals (Fukai et al., 2013). Suitable specimens include vesicular fluid and epithelial tissue homogenates. There have been

recent improvements in the development of more sensitive cell lines for the isolation of FMDV. Such advancements have demonstrated that a continuous bovine cell line can be developed that could stably express both subunits of the $\alpha_v\beta_6$ integrin, the principal cell receptor for FMDV (LFBK- $\alpha_v\beta_6$) (LaRocco et al., 2013). These cells were more sensitive to infection with FMDV from clinical specimens compared to BHK, IB-RS-2 and MVPK-1. Although, cell culture systems can be highly sensitive, it is a relatively slow process to propagate the virus (1-4 days), and the results can be influenced by the quantity and quality of the specimen in addition to host-specificity of certain strains of FMDV (Ludi et al., 2017). Virus isolation requires investment in sterile facilities and techniques and technical expertise for the maintenance of cells and logistics associated with the regular sourcing of certified animals to be FMD-free in the case of BTY. Thus, these limitations make virus isolation inaccessible to most laboratories in resource-scarce settings. Furthermore in most cases, cytopathic effect in the susceptible cells is not sufficient to confirm the diagnosis of FMD (Ludi et al., 2017) and needs to be followed by a confirmatory test such as PCR.

2.11.1.2 Antigen-capture enzyme-linked immunosorbent assay

The antigen-capture sandwich ELISA is a highly sensitive method for virus detection and typing from clinical materials (OIE, 2018). The assay is the test of choice for FMD endemic countries because it can be automated to process large quantities of samples and be completed within a few hours. The test is based on a sandwich ELISA in which plates are coated with serotype-specific rabbit polyclonal sera (coating sera) and virus present in the processed clinical specimen will bind to the capture antibodies. The bound viruses are detected by adding serotype-specific tracing/detecting antibodies followed by a secondary antibody bound to a suitable enzyme. The assay can be used to differentiate FMDV serotypes due to the use of serotype-specific capture and detecting antibodies (Roeder and Le Blanc Smith, 1987). This assay replaced the complement

fixation test for primary FMD diagnosis and serotype identification (Ferris and Dawson, 1988). Sample quality as well as the optimal timing for sample collection is an important factor in this assay, as lesions older than 4-5 days might contain less virus (Ferris and Dawson, 1988). However, specimens unsuitable for virus isolation can still be tested using this assay. The assay can also be used to type and verify FMDV in cell culture supernatants. The assay has a lower analytical sensitivity compared to virus isolation when used for direct detection of FMDV in clinical epithelial specimens (Ferris and Dawson, 1988). However, it has been described to be well suited for low technology settings because it does not require the use of live virus and is based on robust (reproducible) technology.

2.11.1.3 Lateral flow device

Several lateral flow devices (LFD) employing monoclonal antibodies have been developed for the detection of FMDV antigens from clinical specimens (Ferris et al., 2009; OIE, 2018). However, most LFD have not been fully validated for all seven FMDV serotypes. This test is based upon the principles of immuno-chromatography, in which soluble antigens from the clinical specimen can flow through a porous cellulose strip. As the solution passes through the strip it first passes through a zone where it meets and solubilises dried labelled antibody conjugate and forms an immune complex. The fluid then flows through a detection zone containing an immobilised antibody against the antigen. Although, several LFD have been developed for the pen-side detection of FMDV, their accuracy was lower for the detection of SAT serotypes. When a monoclonal antibody (MAb) 1F10 was substituted with a MAb 2H6 in one of the devices, the sensitivity for the detection of FMDV SAT2 was enhanced from 65% to 90% (Ferris et al., 2010). With a specificity of 99.4% and comparable sensitivity of 88.2% for the detection of FMDV SAT2 antigens, this device has been described to be superior to the slower and more complicated antigen capture ELISA (Ferris

et al., 2010). The LFD has also been described to be simple, rapid and easy to perform, making it one of the most user-friendly pen-side tests for the diagnosis and serotyping of FMDV (Ferris et al., 2010; Ferris et al., 2009). LFD variations have been produced, including one that can detect and differentiate serotypes O, A, and Asia-1 (Yang et al., 2015) and another one for the detection of vesicular stomatitis virus (Yang et al., 2013; Ferris et al., 2012; Lin et al., 2011). Furthermore, LFD for FMDV antigen detection in the field can then be used for dry, non-hazardous sample transportation to the laboratory for diagnosis using nucleic acid amplification, sequencing and recovery of infectious virus by electroporation (Fowler et al., 2014).

2.11.2 Diagnostic methods for universal detection of virus genes in clinical specimen

2.11.2.1 FMDV Reverse Transcription-Polymerase Chain Reaction

Molecular biological assays including real-time reverse-transcription polymerase chain reaction (RT-PCR) can be employed for the detection of the FMDV genome (RNA) in clinical specimens. These tests offer the advantages of being fast, sensitive and more robust compared to virus isolation. The amplification of specific nucleic acid sequences using the RT-PCR is now widely used for the detection of FMDV RNA in clinical specimens. Molecular assays are suitable for a diverse range of different specimen types including tissues, blood, swabs, oesopharyngeal fluid, faeces and milk (Ludi et al., 2017). The FMDV VP1-coding region plays an important role in the antigenic and phylogenetic characterisation of FMDV as it contains the major immunogenic sites, including vital amino acid residues (Baranowski et al., 2000; Jackson et al., 2002). However, the VP1-coding nucleotide sequence varies between FMDV serotypes. FMDV has also been classified further into topotypes and lineages based on phylogenetic analysis of the VP1-coding region (Knowles and Samuel, 2003). These genetic variations between FMDV strains is commonly used in tracing the origin and movement of outbreak viruses (Abdul-Hamid et al., 2011; Di Nardo et

al., 2014; Knowles et al., 2016). Currently, a pan-specific real-time RT-PCR for FMDV detection has been the mainstay in most FMD laboratories (Callahan et al., 2002; Moniwa et al., 2007; Reid et al., 2009; Reid et al., 2002). These assays amplify the highly conserved RNA sequences within the 5'-untranslated region (UTR) or the RNA polymerase (3D-coding region). Thus, FMDV can be detected by these assays with high diagnostic sensitivity and specificity, since these genome regions are highly conserved for all seven FMDV serotypes. As the VP1-coding region is known to vary according to FMDV serotypes, FMDV serotyping can be achieved by serotype-specific detection of the VP1-coding region, without the need for virus isolation (Bachanek-Bankowska et al., 2016). Conventional RT-PCR methods targeting the VP1-coding region has also been developed (Alexandersen and Donaldson, 2002; Callens and De Clercq, 1997; Vangrysperre and De Clercq, 1996). Conventional RT-PCR assays that target the conserved regions of the FMD genome 3D and 5' untranslated region (5' UTR) utilised agarose gel electrophoresis for the detection of amplified products (Reid, Ferris et al., 2000; Rodríguez et al., 1994; Meyer et al., 1991). However, this procedure is labour intensive (Reid et al., 2001), and carries a high risk of generating false positive results due to carry-over of PCR amplicons when loading samples (Ludi et al., 2017). The real time RT-PCR assay is an improvement over conventional methods and has now largely replaced the traditional agarose gel-based assay in most laboratories. RT-PCR is a more rapid, fluorescence based, assay that is highly sensitive enabling simultaneous amplification and quantification of FMDV-specific nucleic acid sequences (Callahan et al., 2002; Reid et al., 2002). The benefits of this assay in addition to its improved sensitivity include a reduction in the risk of contamination as well as high throughput applications. Real-time RT-PCR methods including the use of Taq Man probes, molecular beacons, primer probe energy transfer based assays as well as RT-PCR of the 3D gene have been developed for FMD diagnosis and are now

the mainstay of FMD genomic diagnosis (Niedbalski and Kesy, 2010; Oem et al., 2005; Moonen et al., 2003; Callahan et al., 2002; Reid et al., 2002). The comparison of RT-PCR with conventional PCR (Ferris et al., 2006; Shaw et al., 2004) concluded that RT-PCR was generally more analytically sensitive and thus ideal for specimens with expected low virus concentrations. Several RNA detection assays targeting the FMDV genome have been developed over the years using reverse transcription loop-mediated isothermal amplification (RT-LAMP), with some detecting only one serotype and others multiple serotypes (Ding et al., 2014; Kasanga et al., 2014; Madhanmohan et al., 2013; Yamazaki et al., 2013; Chen et al., 2011). RT-LAMP is faster, simpler, more cost-effective and at least as sensitive and specific as RT-PCR (Knight-Jones et al., 2016). RT-LAMP can also be used successfully as a portable (pen-side) platform for the rapid field diagnosis of FMDV infection (Abd El Wahed et al., 2013). An RT-LAMP assay has been coupled with a lateral flow device for use as a rapid, low cost means for the early field detection of FMDV infection (Waters et al., 2014).

2.11.3 Diagnostic methods for serological detection of FMDV antibodies

2.11.3.1 Virus neutralisation test

The virus neutralisation test is a serotype-specific serological test for the detection of FMD antibodies against structural proteins in the serum of vaccinated and infected animals (OIE, 2018). The assay depends on the use of cell culture, live virus and sera. With each test, a virus titration is included to determine the actual virus titre and dose for the test. This assay estimates the ability of antibodies in the serum to neutralise the biological activity of the antigen *in vitro*. If the serum from infected animals contain virus-specific neutralising antibodies, the virus will be prevented from infecting cells after the virus-specific antibodies have bound to the antigen and blocked their critical attachment sites; thus, no cytopathic effect (CPE) will be seen in the cultured cells. Positive

wells where the virus has been neutralised and the cells remained intact contain blue stained cell sheets when viewed microscopically after 48 hours of incubation and staining with methylene blue stain. Negative wells where the virus has not been neutralised will appear empty (OIE, 2018). Serum titres are expressed as the $\log_{10}$ reciprocal of the dilution that protects 50% of the cell culture cells from lysis due to the virus. The test is considered to be valid when the amount of virus used per well is in the range of $\log_{10}$ 1.5 – 2.5 $TCID_{50}$, and the positive standard serum is within two-fold of its expected titre (OIE, 2018). However, test results can vary between laboratories and the assay can have poor repeatability and reproducibility. The assay is used in most FMD reference laboratories to assess the antigenic match between vaccine strains and circulating outbreak FMDV. The use of live virus requires that the assay be performed in bio-containment facility using a biosafety cabinet. This assay is more variable than ELISA because of the use of cell cultures and live virus. Furthermore, the test is time consuming and susceptible to contamination of the cell cultures (OIE, 2018).

2.11.3.2 Liquid-phase blocking enzyme-linked immunosorbent assay

The FMD liquid-phase blocking ELISA (LPBE) is a serotype-specific serological assay developed for the detection of FMDV-specific antibodies against structural proteins in serum of animals exposed to or vaccinated against FMDV (OIE, 2018). The assay is widely applied for either the detection (screening test) or quantification (titration test) of antibodies to one or more FMDV serotypes. Serum antibodies are induced against the outer capsid structural proteins following both FMD vaccination in domestic animals and against infection in both domestic and wild cloven-hoofed animals. This is an OIE recommended test for the establishment of FMD free status in animals destined for import and export trade (OIE, 2018). The assay can detect antibodies against all seven FMDV serotypes using polyclonal rabbit and guinea pig IgG antibodies to detect residual

FMD antigen following *in vitro* incubation of test serum and FMD antigen in a "liquid-phase". The principle for the test is based on the ability of the antibody present in the test serum to block the FMD antigen from subsequent detection. Test results are correlated with virus neutralisation test results (Araújo et al., 1996), and this has been widely applied as an *in vitro* method to estimate protection to FMDV challenge (Hamblin et al., 1987; Hamblin et al., 1986; Robiolo et al., 1995; Smitsaart et al., 1998; Van Maanen and Terpstra, 1989). The LPBE is one of the recommended ELISA methods for the detection of FMDV-specific antibodies (OIE, 2018) and is the primary test for estimating Post-vaccination titres. The test is also employed in FMD reference laboratories to assess the antigenic match between vaccine strains and circulating outbreak FMDV. However, the solid-phase competition ELISA has recently replaced this assay in most laboratories around the world.

2.11.3.3 Solid-phase competition enzyme-linked immunosorbent assay

The solid-phase competition ELISA (SPCE) is another OIE recommended serological test that has been developed as an alternative to the LPBE for the detection of antibodies against structural proteins for all the seven FMDV serotypes (Mackay et al., 2001). The test is based on competition between serotype-specific guinea-pig anti-FMD antiserum and antibodies present in the test serum. It is more rapid than the LPBE because it is performed in a "solid-phase" and results can be obtained in a matter of hours (4-5 h). The SPCE is more robust and 100% sensitive relative to the LPBE (Mackay et al., 2001). This assay has a sensitivity of 100% for FMDV serotypes O, A and C (Mackay et al., 2001) and a specificity of 99% for SAT serotypes (Li et al., 2012). The test has a specificity of 99-100% for the detection of antibodies against the FMD serotypes SAT1-3 (Li et al., 2012). A single spot version of the SPCE allows for the screening of a high volume of samples. As an improvement to the LPBE, the test was used extensively during the UK FMD outbreak to

allow for the rapid screening of serum for FMD antibodies (Paiba et al., 2004; Mackay et al., 2001). Several other ELISA assays have been developed and validated, including commercially available kits e.g. IZSLER Brescia-Italy, type O, A, SAT1 and SAT2, Prionics type O, A and Asia-1 kits.

2.11.3.4 FMDV non-structural protein enzyme-linked immunosorbent assay

Several commercially available tests and in-house assays for the detection of non-structural protein-specific antibody responses have been developed (Ludi et al., 2017). The NSP ELISAs are non-serotype specific assays employed to differentiate serum antibodies induced by FMDV infection from those induced by vaccination (Diego et al., 1997). The detection of FMDV non-structural protein-specific antibodies is the basis of most DIVA (differentiating infected from vaccinated animals) tests (Biswal et al., 2014; Mohapatra et al., 2014; Srisombundit et al., 2013; Gao et al., 2012; Sharma et al., 2012; Jaworski et al., 2011). The assay typically employs ELISA technology but a luminex-based assay has also been developed (Chen et al., 2013). Antibodies against NSP are induced in animals following natural exposure to virus replication. However, the strength of the NSP antibody response in vaccinated animals is variable and depends upon the extent of virus replication. A comparative study of six NSP assays, five that detect antibodies against the 3ABC NSP and one that detects antibodies against the 3B NSP was performed. The ability of these tests to detect vaccinated animals that have been subsequently exposed to FMDV varied considerably from 38% to 78%, although these sensitivity values were higher when only specimens from carrier animals were evaluated (48% to 89%) (Ludi et al., 2017). The specificity of these assays in vaccinated cattle has been reported to exceed 96% (Brocchi et al., 2006). It is recommended that NSP assays be used on younger animals of less than 18 months of age because repeated vaccination, even with highly purified FMD vaccines, can generate false positive results

(Elnekave et al., 2015). NSP ELISAs are not serotype-specific and can be used as a generic screening test for FMDV infection. Following infection with FMDV, NSP sero-conversion usually occurs after 7-14 days but the antibody responses can be detected in serum for months or even years, depending upon the amount of virus replication (Elnekave et al., 2015; Parida et al., 2005; Paton et al., 2009). In a study to validate the performance of a SAT serotype-specific 3ABC assay in cattle, three assays (PrioCHECK®-NSP, IZSLER- NSP and SAT-NSP) detected NSP antibodies at 5-7 days post-infection with SAT1, SAT2 and SAT3 viruses with the exception of SAT1/NIG/5/81 infected animals, which only tested positive at 14 days post-infection (Chitray et al., 2018). Animals with localized infections, with little to no systemic infection might not develop a detectable NSP-specific antibody response, particularly if previously immunized (Brocchi et al., 2006). Therefore, interpretation of serosurveillance data from vaccinated herds can be complex and require a larger number of animals to be tested.

2.12 Social network analysis and its application in disease surveillance and control

The application of social network analysis in the livestock industry has improved our knowledge of the patterns of livestock movement and to inform risk-based surveillance systems (Poolkhet et al., 2019; Dubé et al., 2008). Social network analysis has been used extensively to analyse livestock movements (Mweu et al., 2013; Aznar et al., 2011; Nöremark et al., 2011; Green et al., 2008; Kiss et al., 2008; Robinson et al., 2007; Kao et al., 2006). Social network analysis can identify targets for surveillance, intervention and control (Bajardi et al., 2012; Natale et al., 2009; Kiss et al., 2008; Christley et al., 2005). In social network studies, there are two components to consider: the node and the tie. The node refers to each unit of interest in the study, while the tie refers to the links between the respective units of interest (Borgatti et al., 2013). A network analysis of the UK FMD outbreak (Shirley and Rushton, 2005) identified livestock markets and dealers as the hubs

for disease spread. Movement of cattle through the markets is an important contributor to FMD outbreak spread (Robinson and Christley, 2007). Certain nodes (some farms, animal markets and dealers) were key players in the early spread of FMD during the outbreak (Ortiz-Pelaez et al., 2006). All nodes had a high between-ness centrality, a measure of how frequent a node is located between every pair of node connections. Auction markets were also key players for the spread of disease during the UK FMD outbreak (Robinson and Christley, 2007). A similar study in Denmark also reported that cattle markets influenced other nodes within the Danish cattle movement network (Mweu et al., 2013). The between-ness centrality and the closeness centrality, a measure of how close the node is to other nodes were higher for cattle markets than for any other node. The transportation of infected cattle is a known contributor to disease transmission and the out-degree centrality, a quantification of the number of outgoing ties from the node, is useful for estimating the epidemic size (Dubé et al., 2008). In Italy, network analysis has been applied to evaluate the potential risk of disease spread through the cattle trade network (Natale et al., 2009) and that the control of infectious livestock diseases such as FMD should focus on livestock movements. During FMD outbreaks, strict quarantine and movement control of livestock and livestock products is often imposed in addition to emergency vaccination and enhanced surveillance. Surveillance of infectious livestock diseases constitutes an integral part of an effective disease control and prevention strategy. This can provide vital information for early disease detection, declaration of disease freedom and evaluation of control programmes. Historically, the movement of humans and animals have contributed significantly to the transmission of disease epidemics (Van Kerkhove et al., 2009). The outbreaks of the ancient “Black Death” throughout Europe and Asia were linked to the movement of humans on foot, horseback and boats (Scott, 2005). More recently, the outbreak of the novel coronavirus (COVID-19) has spread rapidly from its origin in Wuhan, Hubei

Province, China and expanded to a pandemic through human movements (Chinazzi et al., 2020; Kraemer et al., 2020). As a result, most countries of the world imposed movement restrictions in the form of social distancing and lockdown in effort to "flatten the curve" of the pandemic. Human mobility was predictive of the spread of the epidemic in China and a full blown pandemic developed within three months of its first occurrence (Kraemer et al., 2020). The movement of sub-clinically infected animals played an important role in disease transmission during the 2001 FMD epidemic in the UK and the highly pathogenic avian influenza (HPAI) outbreaks in the Netherlands, Bangladesh, Cambodia, China, India, Indonesia, Iran, Israel, Japan, South Korea, Mongolia, Myanmar, Nepal, Palestine and Vietnam (Boender et al., 2007; Ortiz-Pelaez et al., 2006; Stegeman et al., 2004).

Many livestock diseases are transmitted through direct contact between animals, and thus between herds through animal movements. This is a reason for many countries in the developed world to register livestock movement and transports in national databases (Anonymous, 2014). Information generated from such databases can be used for surveillance and planning purposes. The rapid analysis of livestock movements can be used to implement effective livestock movement restrictions. There has been a wide application of social network analysis for the evaluation of livestock movements and disease investigations (Dube et al., 2009; Martinez-Lopez et al., 2009). Network analysis became widely applied in the field of veterinary epidemiology after the 2001 FMD outbreak in the UK (Christley et al., 2005; Webb, 2005; Webb, 2006; Kiss et al., 2006; Ortiz-Pelaez et al., 2006; Bigras-Poulin et al., 2006; Bigras-Poulin et al., 2007; Robinson and Christley, 2007; Robinson et al., 2007; Dubé et al., 2008; Frössling et al., 2012; Fasina et al., 2015; Noopataya et al., 2015).

Until recently, livestock movements had been studied using information obtained on the frequency

of animal movements on and off farms (Bates et al., 2001; Christensen et al., 2008; Sanson, 2005; Dubé et al., 2011). However, livestock movement data can also be collected to generate livestock networks. In this situation, the unit of interest is the holding or node, and the relationship is the movement or tie, which produces paths on which infectious disease agents could spread (Dubé et al., 2011). Social network analysis is a powerful tool for study of these networks and to understand the role played by each holding within the network. This provides vital information on holdings that are important in the flow of animals in the population, which could be targeted for surveillance activities to improve early disease detection. This analysis can also help in understanding the potential spread of infectious disease agents during the silent phase prior to detection (Dubé et al., 2011).

CHAPTER THREE

CLINICAL PRESENTATION OF FMD VIRUS SAT1 INFECTIONS IN EXPERIMENTALLY CHALLENGED INDIGENOUS SOUTH AFRICAN GOATS[1]

3.1 Introduction

Foot-and-mouth disease (FMD) is caused by infection with FMD virus (FMDV), a small, positive-sense RNA virus in the genus *Aphthovirus,* family *Picornaviridae* (Han et al., 2018). FMDV infects cloven hoofed species and is classified into seven clinically indistinguishable serotypes (O, A, C, Asia-1 & Southern African Territories (SAT) 1, SAT2 & SAT3). The disease is characterized by fever, lameness and the appearance of vesicular and ulcerative oral and foot lesions (Arzt et al., 2011a; Horsington et al., 2018). Cattle, pigs, sheep and goats are epidemiologically important host species in many parts of the world with sheep having been involved in the spread of infection in numerous outbreaks including the 2001 UK outbreak (Alexandersen et al., 2003a; Anderson et al., 1976; A. Donaldson, 1999; Krystynak and Charlebois, 1987; Samuel et al., 1999; Tsaglas, 1995). Sheep and goats are important livestock species in many areas of the world but they are not typically included in prophylactic FMD vaccination programmes (Madhanmohan et al., 2012, 2011). Experimental studies in cattle, buffalo, sheep and pigs have contributed to our knowledge of the pathogenesis and transmission of FMDV (Alexandersen et al., 2003b; Arzt et al., 2011a; Kinsley et al., 2016; Paton et al., 2018; Stenfeldt et al., 2016).

The clinical signs of FMD in goats are considered to be mild but clinical descriptions for infections with the Southern African Territories (SAT) viruses have not been previously reported (Kitching

[1] SMALL RUMINANT RESEARCH 180 (2019) 15-20

https://doi.org/10.1016/j.smallrumres.2019.09.014

and Hughes, 2002). Antibodies against FMDV non-structural proteins suggestive of viral exposure in unvaccinated animals has been reported previously (Balinda et al., 2009; Bhebhe et al, 2016; Habiela et al., 2010; Hyera et al., 2006; Lazarus et al., 2012). In the Southern African Development Community (SADC), the African buffalo (*Syncerus caffer*) is the wildlife reservoir host maintaining SAT1, SAT2 and SAT3 (Paton et al., 2018; Thomson et al., 2003; Vosloo and Thomson, 2017). FMD outbreaks within the SADC have increased in frequency and in many situations, these outbreaks have persisted for a longer time (Jori et al., 2016; Penrith and Thomson, 2012). Traditional FMD control measures have become inadequate in some parts of the SADC during the last 10-15 years (Lazarus et al., 2018; Thomson et al., 2013; Vosloo and Thomson, 2017). Several countries in the SADC have reported outbreaks during the past decades, with South Africa officially reporting FMD outbreaks within the FMD free zone of the country in February 2011 and January 2019 (DAFF 2019a, 2019; OIE-WAHID, 2017; Vosloo and Thomson, 2017).

The official World Organisation for Animal Health (OIE) recogised FMD free zone status of South Africa has been suspended after detection of a FMDV serotype SAT2 outbreak in the free zone. As a follow up to the recent outbreak, our team identified seropositive sheep and goats within the outbreak area (unpublished data). The control of FMD within the protection zone of South Africa includes routine prophylactic vaccination of cattle with an inactivated trivalent FMD vaccine containing serotypes SAT1, SAT2 and SAT3 (Lazarus et al., 2018). The current paper describes the clinical presentation of FMDV SAT1 infection in experimentally challenged indigenous South African goats.

3.2 Materials and methods

3.2.1 Ethics statement

This study was approved by the University of Pretoria, Animal Ethics Committee (V022-17) and

the Onderstepoort Veterinary Research Animal Ethics Committee (AEC 6.17). Approval in terms of the Animal Disease Act (Act No. 35 of 1984) was obtained from the National Department of Agriculture, Forestry and Fisheries: Directorate of Animal Health, Republic of South Africa.

3.2.2 Preparation of FMD SATI virus pool challenge material

A pool of FMDV SAT1 (SAR/8/10, SAR/10/10 and SAR/21/10) field viruses isolated from cattle during an outbreak within the FMD protection zone of South Africa were propagated in IB RS-2 (swine kidney) and ZZR-127 (foetal goat tongue) mono layer cell lines (Brehm et al., 2009; Chapman and Ramshaw, 1971). A $10^{4.5\text{-}5.5}$ 50% tissue culture infective dose ($TCID_{50}$) of the virus pool was used to inoculate two Boer goats and two Nguni cattle to produce a host-adapted challenge material at two serial virus passages (Sirdar et al., 2019). Clinical material collected from the first pooled virus challenge was used to challenge a second set of two goats and two cattle. Clinical material (epithelial lesions from the mouth and feet) from the second set of goats was again collected and pooled as previously described and prepared as the challenge material for the current study.

3.2.3 Experimental animals

A group of 40 indigenous South African goats (6-12 months of age) were sourced from livestock farms within the FMD free zone of South Africa prior to the 2019 FMD SAT2 outbreak (DAFF, 2019) for the evaluation of an inactivated oil-emulsion FMD vaccine (data not presented). The emphasis of this paper is only the clinical descriptions of the five unvaccinated control goats and two vaccinated unchallenged in-contact goats maintained during the study. The two in-contact goats were vaccinated with a reduced dose (0.33 ml) of the oil-emulsion FMD vaccine containing serotypes SAT1, SAT2 and SAT3.

All challenged and in-contact goats were inoculated intramuscularly in the upper neck region on

day 0 and revaccinated after 20 days. All seven goats were confirmed negative for FMDV-specific antibodies at the start of the study using liquid-phase blocking ELISA for all three SAT serotypes (Hamblin et al., 1986). Pooled FMDV SAT1 clinical material was inoculated into the five challenged goats intra-dermolingually at a dose of $10^{4.57}$ $TCID_{50}$ after sedation with 2% Rompun® (xylazine hydrochloride, Bayer Animal Health). The two vaccinated unchallenged goats were maintained in direct contact with challenged goats for the entire study. Goats were provided with *ad libitum* access to fresh drinking water, fed a complete pelleted ruminant feed once a day and housed at the BSL-3 animal facility, Onderstepoort Veterinary Research, Transboundary Animal Diseases, South Africa.

3.2.4 Clinical scoring

Goats were examined daily with their rectal temperatures and clinical signs recorded. Clinical signs of FMD were scored as previously described (Madhanmohan et al., 2011; Quan et al., 2004) with slight modifications: fever + 1; each secondary lesion away from the site of inoculation + 1. The total clinical score was determined by simple addition and each goat could theoretically score a maximum of 8 points per day: fever, secondary lesions on tongue, gum, lip, and each of four feet. Rectal temperatures ≥40°C were defined as fever (Madhanmohan et al., 2011). All goats were humanely euthanized by intravenous overdose of sodium pentobarbitone (Euthapent®, Kyron Laboratories) 14 days post challenge.

3.2.5 Sample collection and processing

Clotted blood for serology was collected on day 0 before animals were vaccinated and at termination into plain evacuated tubes (Vacutainer®, BD Becton, Dickinson and Company, USA). Samples were allowed to clot at room temperature and sera harvested and stored at -20°C until testing. Heparinised blood was collected at 0, 2, 4- and 6-days post challenge into sodium heparin

(Vacutainer®, BD Becton, Dickinson and Company, USA) for virus detection and stored at -70°C until testing. Epithelial tissue from fresh lesions were collected into a specimen bottle with Roswell Park Memorial Institute (RPMI) 1640 media (Sigma-Aldrich) and stored at -70°C until testing. Oropharyngeal specimens were collected from all goats at 6 days post challenge using a small ruminant probang cup and samples stored in RPMI-1640 media (Sigma-Aldrich) at -70°C until testing. Briefly, after the application of physical restraint on the goats, a small ruminant probang cup was carefully inserted into the oro-pharyngeal area passing it over the tongue and moving it vigorously backwards and forwards 5 – 10 times between the first portion of oesophagus and the back of the pharynx (OIE, 2018). The cup was removed carefully and the contents poured into a wide-necked transparent sample bottle containing transport media and antibiotics.

3.2.6 Laboratory analysis of specimens

3.2.6.1 Solid phase competition ELISA (SPCE)

A SPCE for FMDV serotype SAT1 was performed following standard procedures (Paiba et al., 2004; Mackay et al., 2001). Tests were performed in duplicate and optical density (OD) values averaged. The final OD were expressed as the percentage inhibition (PI) relative to the mean OD of four strong positive control wells. i.e. 100 – (100 x (OD test serum mean/OD strong positive control mean)). Samples that showed <50% inhibition of the OD strong positive control were classified as negative and those ≥50% were considered a positive serological response (Paiba et al., 2004). SPCE is a serotype-specific serological assay with a relative sensitivity between liquid-phase blocking ELISA and virus neutralization tests of 100% respectively using SAT1-3 (Li et al., 2012) and a sensitivity of 100% for FMDV serotypes O, A and C (Mackay et al., 2001). The test has a specificity of 99% for SAT serotypes (Li et al., 2012).

3.2.6.2 Quantitative real-time RT-PCR

Real-time RT-PCR was performed on heparinized blood, epithelial tissues and oropharyngeal specimens collected from all animals. Total cellular RNA was extracted using the QIAamp® Viral RNA kit (Qiagen, Hilden, Germany) or the TRIzol™ (Invitrogen, USA) following the manufacturer's instructions. Real-time RT-PCR was conducted using iTaq™ Universal Probes One-Step kit (Bio-Rad, CA, USA) according to the manufacturer's instructions. Primers targeting the FMDV 3D region were sense 5'-ACT GGG TTT TAC AAA CCT GTG A-3' and antisense 5'-GCG AGT CCT GCC ACG GA-3'. The probe was 5'-TCC TTT GCA CGC CGT GGG AC-3'; its 5' end was labeled with 6-FAM, and the 3'end was labeled with TAMRA (Callahan et al., 2002). The CFX96™ Real-Time PCR Detection system (Bio-Rad, CA, USA) was used for virus detection. Specimens with a cycle threshold value ≤35 were considered positive.

3.3 Results

3.3.1 Clinical outcomes

All five goats challenged with the FMDV SAT1 pool developed elevated temperatures within 48 hours with a median fever duration of 5 days (Figure 3.1). One goat (L26) had fever that lasted for 10 consecutive days. Four goats had tongue lesions at the site of inoculation 72 hours post challenge (Figure 3.2, top left). Animal L10 developed a tongue lesion two days post challenge and presented with bilateral nasal discharge on day 3, which lasted for three days (Figure 3.2, top right). Animal 166 developed a tongue lesion on day 4 at the site of inoculation and a secondary lesion of the ventral oral lip on day 7 (Figure 3.2, bottom left). Animal L26 developed a tongue lesion on day 2, nasal discharge on day 3 and left front hoof and right front hoof inter-digital cleft lesions on day 8. Animal L7 developed a tongue lesion on day 2 and a right hind limb hoof lesion on day 6 (Figure 3.2, bottom right). Animal L17 only developed a tongue lesion on day 2 post

challenge. One of the vaccinated unchallenged in-contact goats (L28) developed fever on in-contact day 7, which lasted for three consecutive days. The other goat (161) developed fever on in-contact day 5 that only lasted for 1 day (Figure 3.3). Animal 161 developed an ulcerative lesion on the lip at in-contact day 4 and the other goat (L28) developed a similar lip lesion on day 8. The maximum clinical score for the challenged goats was three on days 8, 9 and 10 post-challenge (Table 3.1). Clinically apparent lameness was not identified and none of the goats lost weight or had a reduced appetite at any time during the study.

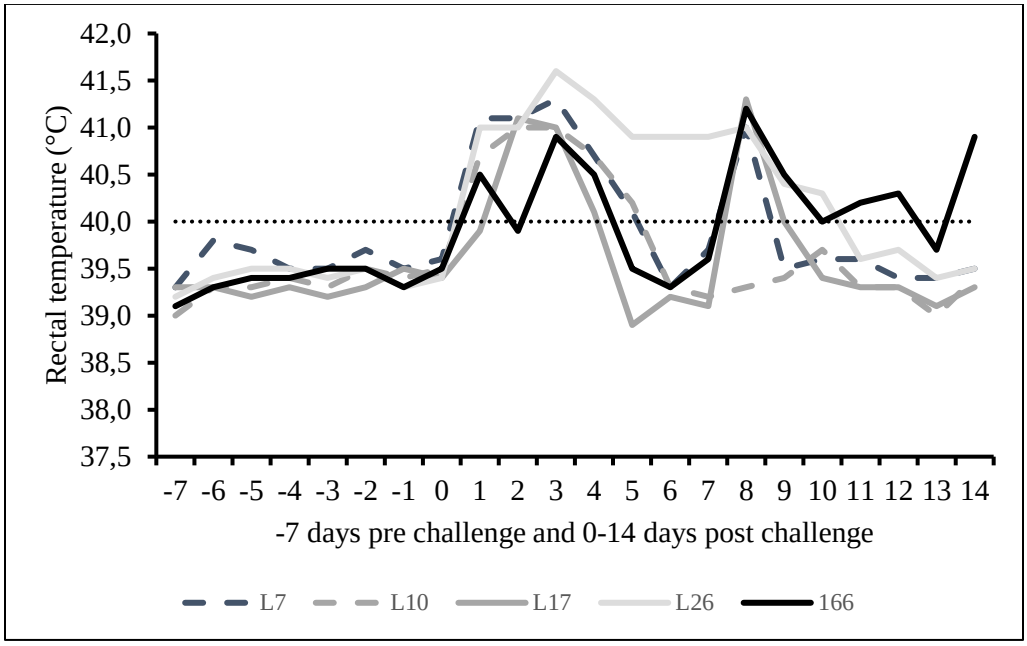


Figure 3.1 Rectal temperature -7 days pre challenge to 14 post challenge of goats intra-dermolingually inoculated with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool. Probang samples (oropharyngeal specimen) were collected day 6 post challenge. Fever was defined as a temperature ≥40°C.

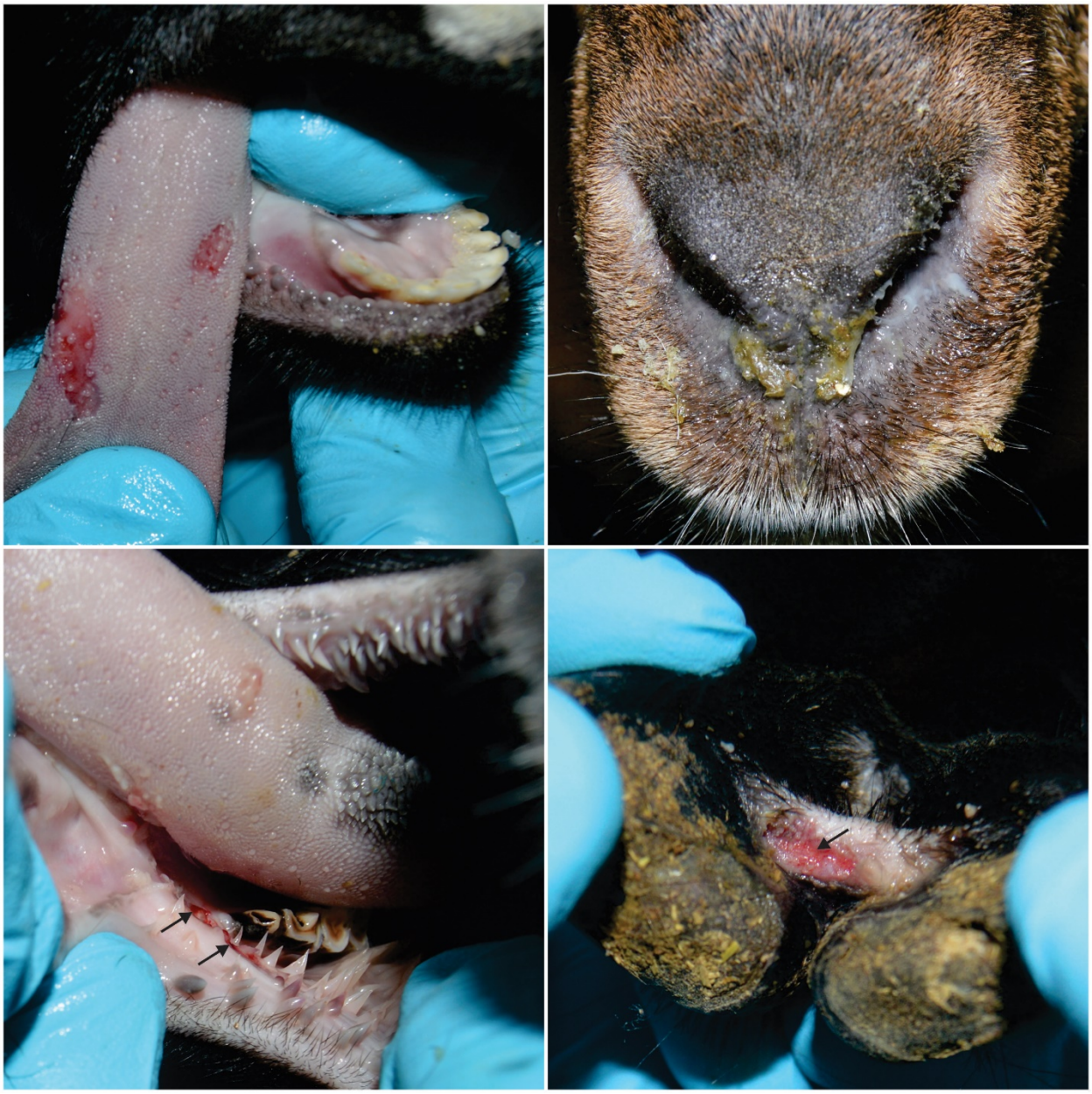


Figure 3.2 Top left: Ulcerative tongue lesion at the site of inoculation 48h post challenge of an indigenous South African goat with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool. Top right: Bilateral nasal discharge 3 days post challenge. Bottom left: Ulcerative lesion on the oral mucosa of the ventral lip. Bottom right: Inter-digital cleft lesion 6 days post challenge.

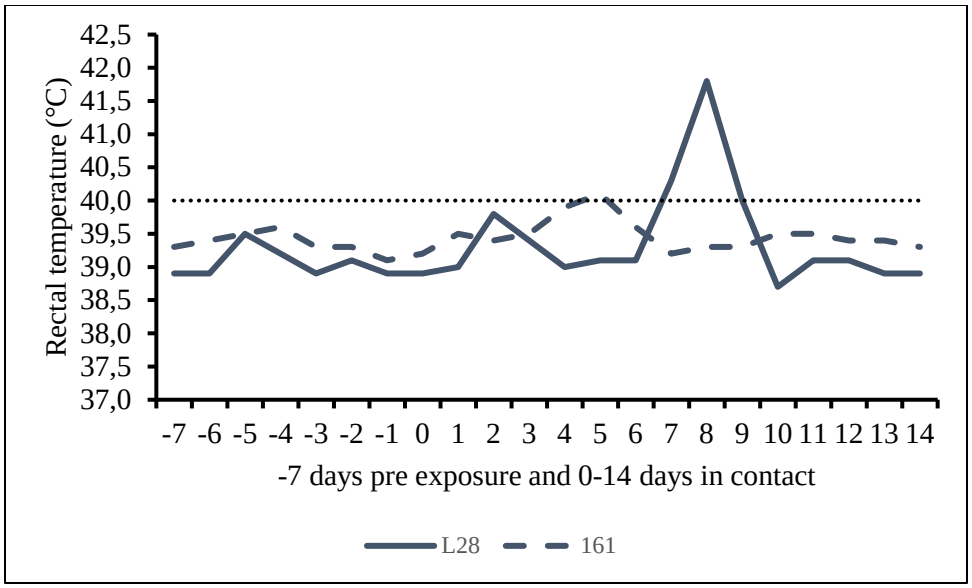


Figure 3.3 Rectal temperature -7 days pre-exposure and 0-14 days in contact for the two unchallenged goats maintained with the challenged goats. Fever was defined as a temperature ≥40°C.

Table 3.1 Clinical lesion scores of five goats following intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool and two unchallenged goats maintained in direct contact with experimentally infected goats.

Group.	Goat	0 dpc	1 dpc	2 dpc	3 dpc	4 dpc	5 dpc	6 dpc	7 dpc	8 dpc	9 dpc	10 dpc	11dpc	12 dpc	Total
Experimentally infected	L7	0	1 [F]	1 [F]	1 [F]	1 [F]	1 [F]	1 [RH]	1 [RH]	2 [F, RH]	1 [RH]	1 [RH]	1 [RH]	0	12
	L10	0	1 [F]	1 [F]	1 [F]	1 [F]	1 [F]	0	0	0	0	0	0	0	5
	L17	0	0	1 [F]	1 [F]	1 [F]	0	0	0	1 [F]	1 [F]	0	0	0	5
	L26	0	1 [F]	1 [F]	1 [F]	1 [F]	1 [F]	1 [F]	1 [F]	3 [F, LF, RF]	3 [F, LF, RF]	3 [F, LF, RF]	2 [LF, RF]	0	18
	166	0	1 [F]	0	1 [F]	1 [F]	0	0	1 [L]	2 [F, L]	2 [F, L]	1 [F]	1 [F]	1 [F]	11
In-contacts	L28	0	0	0	0	0	0	0	1 [F]	2 [F, L]	2 [F, L]	1 [L]	0	0	6
	161	0	0	0	0	1 [L]	2 [F, L]	1 [L]	0	0	0	0	0	0	4

dpc – days post challenge, Individual clinical signs were recorded as follows: fever – +1; each secondary lesion away from the site of inoculation – +1; individual lesion on the hoof – +1. F – fever, L – oral mucosa ventral lip, LF – left front limb, RF – right front limb, RH – right hind limb. The scores were then added. Since development of lesions at the site of inoculation was not considered indicative of generalization of disease, it was not scored. A goat could therefore score a maximum of 8 points.

3.3.2 Antibody responses

SPCE against SAT1 viruses were negative in all goats at the beginning of the study with a mean ± SD percentage inhibition (PI) of 5 ± 6 (Table 3.2). At termination (55 days) of the study, all goats were FMDV SAT1 seropositive with a mean ± SD PI of 83 ± 7.

Table 3.2 Solid phase competition ELISA (SPCE) percentage inhibition (PI) values for five goats experimentally infected with a pool of FMD SAT1 viruses and two in-contact exposed goats at the beginning and termination of the study.

		Day of infection (d0)		Day of termination (d55)	
Group	Goat ID	PI	Interpretation	PI	Interpretation
Experimentally infected	L7	8	Negative	77	Positive
	L10	8	Negative	86	Positive
	L17	16	Negative	75	Positive
	L26	5	Negative	79	Positive
	166	-2	Negative	81	Positive
In-contact	L28	4	Negative	91	Positive
	161	-1	Negative	91	Positive

Day 0 = inception of trial, Day 55 = termination of trial, SPCE PI threshold ≥50% = positive

3.3.3 Virus detection

All lesion materials (epithelial tissues) collected from challenged goats tested positive for FMDV RNA by RT-PCR (Table 3.3). However, only one sample of epithelial material tested positive from the two in-contact goats. FMD viral RNA was detected in heparinized blood sample of three challenged goats at 2 days post challenge and two goats at 4 days post challenge. All animals were positive for viral RNA in oropharyngeal specimens.

Table 3.3 Foot-and-mouth disease (FMD) viral detection in clinical specimens as determined by RT-PCR after challenge with FMDV SAT1 pool (in challenged goats) and unchallenged in-contact goats.

DPC		1	2	3	4	5	6	7	8
Group	ID								
Experimentally Infected	L7	-	$E^+ B^+$	-	-	-	$E^+ O^+$	-	E^+
	L10	-	$E^+ B^+$	E^+	B^+	-	O^+	-	-
	L17	-	E^+	-	-	-	O^+	-	-
	L26	-	$E^+ B^+$	-	B^+	E^+	O^+	-	-
	166	-	-	-	E^+	-	O^+	-	-
In-contacts	161	-	-	-	E^+	-	O^+	-	-
	L28	-	-	-	-	-	O^+	-	E^-

DPC= days post challenge, E^+ = epithelial tissue positive for viral RNA, E^- = epithelial tissue negative for viral RNA, B^+ = blood positive for viral RNA (viraemia), O^+ = oropharyngeal specimen positive for viral RNA, All specimens were tested for FMDV RNA by RT-PCR

Epithelial tissues were collected as they appeared, blood for viraemia was collected on 0, 2, 4 and 6 dpc and oropharyngeal specimens were collected at 6 dpc.

3.4 Discussion

The clinical signs observed in this study were consistent with what has been reported for sheep (Zaikin, 1959; Littlejohn, 1970; Kitching and Hughes, 2002). The most prominent signs were fever, ulcerative oral and hoof lesions. The second peak in rectal temperatures in all challenged animals followed oropharyngeal sampling on day 6 post challenge, which was likely associated with the stress of sedation and animal handling. The two vaccinated unchallenged goats maintained during the study only developed oral lip lesions following natural transmission via direct contact. This was similar to our field observations where ulcerative oral lesions were observed in goats during the recent South African SAT2 outbreak. This outbreak in cattle was confirmed by RT-PCR but virus was not detected in the sampled goats. Only serological evidence of FMDV exposure was identified in sampled small ruminants (data not presented). In both the epithelial tissues of the goat observed from the field and one of the vaccinated unchallenged goats that had lip lesions, no viral RNA was detected in the specimens even though the lesions were consistent with FMD. There seems to be no biological explanation as to why the two specimens tested negative by RT-PCR, while the rest of the specimens tested positive using the same assay.

Importantly, one of the vaccinated unchallenged goats developed a lip lesion before manifesting fever. This suggests viral shedding might have occurred before the appearance of clinical signs. This finding is consistent with a previous study suggesting that fever is not a reliable predictor of FMD generalization in sheep (Horsington et al., 2015). The short duration of fever and mild clinical lesions in the vaccinated unchallenged goats might have been a result of the dampening effect of the vaccine. FMD vaccination does not induce sterile immunity (Horsington et al., 2018; Lyons et al., 2016); however, vaccination can reduce viral shedding and clinical signs in most cases (Horsington et al., 2015; Parida et al., 2008). This is the rationale for prophylactic

vaccination in endemic settings (FAO, 2016).

The clinical signs of FMD appeared in both the challenged and unchallenged goats between 4-8 days. This is similar to previous reports of a 2-8 day FMD incubation period in sheep and goats (Kitching and Hughes 2002; McVicar and Sutmoller 1972). However, this variation in timeline might depend on susceptibility of the host species, challenge virus dose as well as the route of infection. Infection with this pooled mixture of FMDV SAT1 only caused mild clinical lesions in our study goats. Clinical findings were classified as mild since the goats did not become anorexic or lame and observed lesions were less severe than what has been typically reported for cattle and sheep. We are uncertain if goat breed or the administered SAT1 FMDV pool of viruses influenced the clinical presentation in our study animals. Viraemia typically occurs 24-30 hours following intranasal inoculation in sheep and lasts for 1-5 days (Hughes et al., 2002). As in cattle and pigs, fever and vesicles have been described to be the hallmark of clinical FMD in small ruminants and this has been reported to occur within 12-48 hours after the onset of viraemia (Arzt et al., 2011). This is similar to the results of the present study where viraemia was detected 2-4 days post challenge with clinical signs appearing after the viraemic phase. Also, aerosol shedding of the virus in sheep reaches a peak before the onset of clinical signs (Alexandersen et al., 2002; Burrows, 1968).

Some experimentally challenged goats developed nasal discharge, which has not been previously reported. However, following infection, FMDV replicates within the pharyngeal tissues and there is evidence that primary replication might occur in the nasal mucosa of sheep (Arzt et al., 2011). Oral lesions might occur more commonly in goats relative to sheep with some strains of FMDV (Olah, 1976); however, in field outbreaks affecting both sheep and goats, clinical signs are often reported to be more mild in the later (Arzt et al., 2011). One goat that presented with clinical signs

before the development of fever also suggests that sub-clinically infected goats might shed virus silently without obvious signs of disease. Consequently, when inspecting goats for suspected FMD infections, attention should be focused on the oral mucosa of the lips and gums in addition to the tongue. For improved diagnostics, there is the need to further evaluate the performance of the RT-PCR for the detection of FMDV clinical specimens in goats. There is also a need to investigate the role of goats in the epidemiology and maintenance of FMDV under field conditions in southern Africa.

This was a small experimental animal challenge study performed as a part of a study to evaluate vaccine efficacy (data not presented) and results are limited by the small number of animals. However, presented results improve our knowledge of the clinical presentation of SAT1 FMDV infections in goats after experimental challenge and natural transmission. Another limitation of the study is the use of vaccinated unchallenged goats instead of naïve goats for the evaluation of natural transmission. We are also unable to present viraemia data for the unchallenged goats even though we had data on fever and clinical presentations. The research is ongoing, and we are currently uncertain which of the viruses in the pool were responsible for disease. Future genetic evaluation of recovered viruses is a part of the research programme and these findings should answer this question. Continued research is necessary because an understanding of the epidemiological role of non-cattle livestock will improve the progressive control of FMD in southern Africa.

CHAPTER FOUR

EFFICACY OF A FOOT-AND-MOUTH DISEASE VACCINE AGAINST A HETEROLOGOUS SAT1 VIRUS CHALLENGE IN GOATS[2]

4.1 Introduction

Foot-and-mouth disease (FMD) is an acute, highly infectious and economically important transboundary animal disease that affects cattle, buffalo, pigs, sheep and goats (Grubman and Baxt, 2004). The disease is caused by infection with FMD virus (FMDV), a small positive sense RNA virus in the genus *Aphthovirus,* family *Picornavirdae* (Han et al., 2018). Seven clinically indistinguishable serotypes of FMDV have been identified, namely, O, A, C, Asia-1 and Southern African Territories (SAT) 1, SAT2 and SAT3. Among these, serotypes O, A, C, SAT1, SAT2 and SAT3 have occurred in Africa, with serotype C last reported in Kenya in 2004 (Sangula et al., 2011). In southern Africa, FMDV serotypes SAT1, SAT2 and SAT3 are endemic in the African buffalo (*Syncerus caffer*), with sporadic outbreaks of SAT1 and SAT2 occurring in livestock (Brito et al., 2016; Thomson et al., 2003; Vosloo and Thomson, 2017). The disease is characterized by fever, lameness and the appearance of vesicular and ulcerative lesions in the mouth, tongue, nose, feet and teats of lactating animals (Arzt et al., 2011b, 2011a; Horsington et al., 2018). In goats, the clinical signs of FMD are typically considered mild or inapparent (Kitching and Hughes, 2002). However, experimental infection can cause fever, nasal discharges, and development of ulcerative oral and interdigital cleft lesions (Lazarus et al., 2019).

[2] VACCINE 38 (2020) 4006-4015

https://doi.org/10.1016/j.vaccine.2020.04.014

Vaccination is an efficient and cost-effective method of infectious disease control in both human and animal populations (Keeling et al., 2003). However, a successful vaccination programme requires that the vaccine be of high quality and efficacious (FAO, 2016). The overall goal of vaccination in the control of FMD can be broadly classified into four categories: reduction of clinical disease, elimination of circulating virus, maintenance of freedom from disease and regaining freedom from disease (FAO, 2016).

FMD vaccines are biological formulations containing one or more chemically inactivated cell-culture derived seed virus strain preparations blended with a suitable adjuvant and excipients (OIE, 2018). Conventional FMD vaccines are formulated as either aqueous or oil-based preparations (Doel, 2003). An aqueous vaccine, which is mostly used in ruminants, is prepared by adsorbing the virus on to aluminum hydroxide gel and saponin. Oil-adjuvant vaccines are usually formulated using mineral oils (Cloete et al., 2008). While oil-adjuvants are in different forms, the FMD vaccines produced are mainly of the water-in-oil-in-water (W/O/W) form, which are generally formulated using Montanide ISA206 (Seppic, Paris, France) (Park, 2013). FMD vaccines can be classified as either “standard” or “high” potency. Standard vaccines are formulated to contain sufficient antigen to ensure the minimum potency, typically at least 3 PD_{50} (50% protective dose). High potency vaccines (>6 PD_{50}) are formulated with an increased amount of antigen to provide more rapid onset of immunity and a wider spectrum of immunity against closely-related field strains (OIE, 2018).

FMD vaccines are typically developed for use in cattle (Doel, 1996). Vaccines are usually evaluated either by performing live animal challenge studies or by studying serological conversion, which correlates with protection in susceptible species (OIE, 2018). Vaccine efficacy is the ability of a vaccine preparation to protect against disease, virus replication, virus shedding or virus

transmission under controlled conditions (FAO, 2016). SAT FMD vaccines have been evaluated for use in cattle and small stock (Cloete et al., 2008; Hunter, 1998, 1996; Maree et al., 2015). Sheep and goats vaccinated with a trivalent (SAT1, SAT2 and SAT3) oil-adjuvant vaccine maintained humoral antibody levels >1.6 $\log_{10}$ titres for up to 240 days for all three SAT antigens (Hunter, 1996). In a 1982 Nigerian study, cattle vaccinated with a trivalent (SAT1, SAT2 and A) vaccine were protected against a homologous intra-dermolingual challenge at 21 days post-vaccination (Preston et al., 1982). Another study reported that an intra-serotype SAT2 chimeric FMD vaccine could induce strong neutralizing antibody titres that correlated with protection against homologous intra-dermolingual FMDV challenge in cattle (Maree et al., 2015). SAT2 antigen from a thermo-stable and wild-type SAT2 oil-adjuvant vaccine induced full protection (absence of generalized FMD lesions) in all vaccinated cattle following a homologous intra-dermolingual challenge 5 months post-vaccination (Scott et al., 2017). An inactivated FMD vaccine was 80% effective clinically after a homologous SAT2 virus challenge in pigs, with no virus shedding occurring (Mouton et al., 2018). A high potency O_1 Manisa vaccine provided clinical protection following a homologous challenge in goats (Madhanmohan et al., 2012). The same authors reported that one-half of the cattle dose of an oil-adjuvant vaccine is sufficient to induce protective immune responses in goats (Madhanmohan et al., 2011, 2010b). Other successful vaccination studies using FMDV serotypes O, A, C and Asia-1 have also been reported in goats (Madhanmohan et al., 2009; Park et al., 2014; Patil et al., 2002). Including goats in regular vaccinations during outbreaks might improve the control of disease by reducing therisk of subclinical infections and transmission to disease free areas. The objective of the present study was to determine the efficacy of an inactivated double oil-emulsion FMD vaccine in indigenous South African goats challenged with a heterologous pool of SAT1 FMDV.

4.2 Materials and methods

4.2.1 Study design

The study was designed as a blinded randomized control study. Forty FMD sero-negative, indigenous South African goats (6-12 months of age) of mixed sexes were obtained from the FMD free zone of South Africa. Goats were stratified by sex and farm source and randomly allocated to one of five treatment groups using a computer-generated random number list. The calculated sample size for the study was 50 goats based on 80% power and 5% significance for the assumption of a response difference of 0.4 $\log_{10}$ titre values between vaccinated groups and a standard deviation (SD) of 0.3 for the control group (Lazarus et al., 2018). However, considering the need for humane experimentation employing the concepts of the 3 Rs (replacement, reduction and refinement), the G1 (full dose) and the G5 (control groups) were reduced to 5 goats each and the final sample size was therefore 40 goats in total. The sample size was calculated based on the following formula (Chan, 2003).

$$m \text{ (size per group)} = \frac{2c}{\delta^2} + 1$$

Where $\delta = \frac{(\mu_2 - \mu_1)}{\sigma}$ is the standardised effect size and

μ_1 and μ_2 are the means of the two treatment groups

σ is the common standard deviation

c = 7.9 for 80% power, where c is a constant value for 80% power for RCT sample size

From the above, $\delta = 0.4/0.3 = 1.3$

And for 80% power, we had:

m (size per group) = (2 x 7.9)/(1.3 x 1.3) + 1 =10

Five goats were randomly allocated to the G1 full cattle dose (2 ml) and ten goats each to the reduced-dose treatment groups (G2=1/3rd, G3=1/6th and G4=1/12th), and five goats to the G5 unvaccinated control (2 ml placebo) group. The vaccine dose regimen was selected based on recommended dose of 1/3rd cattle dose of alhydrogel-saponin and ½ cattle dose oil-emulsion FMD vaccine preparation for goats (Doel, 2003; Madhanmohan et al., 2010a). Animals were identified by unique identification ear-tags and allowed to acclimatize for 10 days in the BSL-3 animal facility at the Onderstepoort Veterinary Research, Transboundary Animal Disease, (OVR-TAD), Pretoria, South Africa, prior to the study. During the acclimatisation period, all animals were treated with 1% Noromectin® (Norbrook Laboratories, South Africa) at a dose of 5 mg/25 kg subcutaneously and Hi Tet 200 LA Gold® (Bayer Animal Health, South Africa) at a dose of 20 mg/kg deep intramuscularly. Some goats that were affected with infectious keratoconjunctivitis were treated with penicillin topically and Nuflor® (MSD Animal Health, South Africa) at 200 mg/kg intramuscularly for three consecutive days and repeated one week later. Animals were obtained from multiple sources with the possibility of passing through auctions prior to purchase and apparently arrived during the incubation period for respiratory and ocular infections. Animals were provided with *ad libitum* access to fresh drinking water, fed a complete pelleted ruminant ration once a day and housed in the BSL-3 animal facility at the OVR/TAD, Pretoria. All experimental protocols were reviewed and approved by the relevant authorities (AEC V022-17, University of Pretoria and AEC 6.17, Onderstepoort Veterinary Research). Permission for research in terms of the Animal Disease Act, of the Republic of South Africa (Act No. 35 of 1984) was also obtained (DAFF 12/11/1/1, Department of Agriculture, Forestry and Fisheries).

4.2.2 Vaccine administration

The vaccine used was a complete blend of a high potency (>6 PD_{50} in 2 ml cattle dose) pentavalent vaccine containing SAT1 (SAR/9/81/1, BOT/1/106/1), SAT2 (KNP/1/10/2, SAR/3/04/2) and SAT3 (KNP/10/90/3) FMDV strains formulated with Montanide ISA 206 VG™ adjuvant (Seppic, France). The vaccine potency test was performed in a group of healthy FMD antibody-free cattle according to the OIE Terrestrial Manual (OIE, 2018) and the PD_{50} was calculated according to the Reed and Müench method (Reed and Muench, 1937). The vaccine was produced by the OVR-TAD, South Africa for local field use. Goats were vaccinated on day 0 after the initial 10-day acclimation period and revaccinated on day 20 post initial vaccination. The vaccine was administered by intramuscular injection of the left prescapular musculature using individual syringes and 18 G x 1-inch needles. Goats in the unvaccinated control group were administered 2 ml of antigen-free adjuvant as placebo *in lieu* of the vaccine preparation. One researcher not involved in clinical data collection (GTF) administered the vaccine and placebo and all researchers involved in data collection were blinded to treatment group assignment. Animals were housed in separate biosecure animal stables according to treatment group assignments.

4.2.3 Preparation and administration of challenge material

The challenge virus was a pool of three SAT1 (SAR/8/10/1, SAR/10/10/1 and SAR/21/10/1) FMDV isolated during a single outbreak in cattle within the FMD control zone of South Africa during 2010. Virus from field specimens were isolated in IB RS-2 (swine kidney) monolayer cell lines (Chapman and Ramshaw, 1971). The challenge virus was prepared and adapted within two serial animal passages. For each viral passage, two goats and two Nguni cattle were inoculated with the pool of the FMDV SAT1 at a dose of $10^{4.5\text{-}5.5}$ $TCID_{50}$/ml. The challenge material for the second passage was prepared as a pool collected from both cattle and goats due to insufficient

material from goats during the initial passage. The resultant lesion material from the goats only was used for challenging the goats in this study. The preparation of the host-adapted challenge material has been previously described (Lazarus et al., 2019; Sirdar et al., 2019).

The second host-adapted passage SAT1 FMDV pool was used as challenge virus for the current study and administered on day 41 post initial vaccination. After physical restraint and sedation with 2% Rompun® (xylazine hydrochloride, Bayer Animal Health), 34 of the 40 goats were challenged with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool by intra-dermolingual inoculation on the dorsal surface of the tongue (in two sites, total of 1 ml). The six goats that were not challenged were due to losses during the study (n = 3) and the inclusion of partially challenged (n = 1) and unchallenged (n = 2) in-contact sentinels in G3.

4.2.4 Clinical scoring and specimen collection

All goats were monitored for 41 days post initial vaccination concerning their general health prior to challenge. Goats were physically examined daily post-challenge for signs of FMD and rectal temperatures recorded (temperatures ≥40°C defined as fever). The presence of vesicles and ulcerations on the tongue, lips, gums and feet in infected animals were recorded. Clinical signs of FMD were scored as previously described (Madhanmohan et al., 2011; Quan et al., 2004), with slight modifications: fever +1; each secondary lesion away from the site of inoculation +1. The total clinical score was determined by simple addition and each goat could theoretically score a maximum of 8 points per day: fever, secondary lesion on the tongue, gum, lip and each of the four feet.

Blood was collected from the jugular vein into plain evacuated tubes (Vacutainer®, BD Becton, Dickinson and Company, USA) before vaccination and thereafter on a weekly basis until the end of the study. Following challenge at day 41 post initial vaccination, oral, nasal and rectal swab

specimens were collected from all animals on days 0, 2, 4 and 6 post-challenge using Puritan UniTranz-RT™ transport system (Puritan Diagnostics, USA). Blood was allowed to clot at room temperature and collected sera were stored at -20°C until testing. Swab specimens for FMDV RNA detection were stored at -70°C until testing. All goats were humanely euthanized by intravenous overdose of sodium pentobarbitone (Euthapent®, Kyron Laboratories) 14 days post-challenge. The FMD clinical descriptions of goats in this study have been presented elsewhere (Lazarus et al., 2019).

4.2.5 Serological assays

A solid-phase competition ELISA (SPCE) for FMDV serotype SAT1 was performed on collected serum samples following standard procedures (Mackay et al., 2001; Paiba et al., 2004). Tests were performed in duplicate and final optical density (OD) values were expressed as the percentage inhibition (PI) relative to the mean OD of the strong positive control wells. i.e. 100 – (100 x (OD test serum mean/OD strong positive control mean)). Samples with <50% inhibition were scored as negative and those ≥50% were considered positive (Paiba et al., 2004).

Serum samples from 7- and 14-days post-challenge (euthanasia) were tested for the presence of antibodies against FMDV 3ABC non-structural proteins (NSP) using the PrioCHECK® FMDV NS (Prionics, Lelystad, Netherlands). Samples with PI<50% were classified as negative (antibodies against NSP considered absent) while samples with PI≥50% were classified as positive (Sorensen et al., 1998).

4.2.6 Real-time RT-PCR

FMDV RNA was extracted from clinical specimens using the QIAamp® RNA Viral Mini kit (Qiagen, Hilden, Germany) or the QIAamp® RNeasy Mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Real-time reverse transcriptase PCR (RT-PCR) for the

detection of FMDV RNA in oral, nasal and rectal swab specimens was carried out using the iTaq™ Universal Probes One-Step Kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Primers targeting the 3D polymerase region of the FMDV genome were used; 3D forward (5' – ACT GGG TTT TAC AAA CCT GTG A – 3') and 3D reverse (5' – GCG AGT CCT GCC ACG GA – 3'). The probe was 3D probe (6-FAM 5' – TCC TTT GCA CGC CGT GGG AC – 3' TAMRA), (Callahan et al., 2002). The CFX96™ Real-Time PCR Detection system (Bio-Rad) was used and specimens with a cycle threshold ≤35 were considered positive.

4.2.7 Sequences analysis of FMD SAT1 challenge viruses

SAT1 challenge viruses (SAR/8/10/1, SAR/10/10/1 and SAR/21/10/1) were characterized according to RT-PCR and sequencing procedures described previously (Bastos et al., 2003; Blignaut et al., 2020). Nucleotide sequences were submitted to the NCBI GenBank under the accession numbers MT227872, MT227873 and MT227874.

Partial VP1 nucleotide sequences were compiled in multiple sequence alignments using BioEdit v7.2.5 software (Hall, 1999) and CLUSTAL W (Thompson et al., 1994). A Neighbor-joining phylogenetic tree employing the p-distance method was constructed and visualised in MEGA5 (Tamura et al., 2007) for the SAT1 challenge viruses and vaccine reference viruses. For evolutionary analysis, bootstrap values of the phylogenetic nodes were calculated out of 1000 replicates. The evolutionary divergence between SAT1 viral sequences was determined by the comparison of the number of base substitutions per site and was assessed in MEGA5 using pairwise analysis.

4.2.8 Statistical analysis

The normality assumption for all quantitative outcome variables was assessed by calculating

descriptive statistics, plotting histograms and performing the Anderson-Darling test for normality using MINITAB Statistical Software, Release 16 (Minitab Inc, USA). SPCE antibody levels were presented as mean ± standard deviation and comparisons performed using one-way ANOVA. Correlation between SPCE mean PI (7, 14, 20, 27, 34, 41, 48 and 55 dpv) and percentage clinical protection at the group level was estimated by Spearman's rho. The Kruskal-Wallis test was used to compare clinical scores across vaccine treatment groups. Rectal temperatures post challenge was compared among treatment groups using one-way ANOVA with Bonferroni correction of P values for multiple post-hoc tests. Quantitative variables were also compared at each day post-challenge using one-way ANOVA with multiple post-hoc tests adjusted using Bonferroni correction. Linear mixed models were fit to estimate the effect of treatment group on the quantitative outcomes of rectal temperature and viral RNA estimated from real-time RT-PCR (Ct value). Independent models were fit for each outcome in addition to a combined model for the three PCR specimens combined (nasal, rectal and oral swabs). All models included a random effect term for animal with a first-order autoregressive (AR1) correlation structure to account for the repeated measurements. Fixed effects included terms for treatment group and days post challenge (dpc). Bonferroni correction was used to adjust P values for multiple post-hoc comparisons. Statistical analyses were performed in commercially available software (IBM SPSS Statistics Version 24, International Business Machines Corp., Armonk, New York, USA) and significance was set at $P<0.05$.

4.3 Results

4.3.1 Sequence analysis of FMD SAT1 challenge viruses

The SAT1 challenge viruses (SAR/8/10/1, SAR/10/10/1 and SAR/21/10/1) clustered with the SAT1 vaccine strains (SAR/9/81/1 and BOT/1/06/1) (Supplemental Figure 4.1) with average

nucleotide identities of 82.1% and 76.4%, respectively.

4.3.2 Descriptive and clinical results

Forty goats were allocated to the five treatment groups at the beginning of the study with five goats for the G1 (full cattle dose) and ten goats each for the reduced-vaccine groups G2, G3 and G4 and five goats for the G5 (unvaccinated placebo) control.. Thirty-nine goats were vaccinated at the beginning of the study as one goat from the G4 died -6 dpv due to a pre-existing health condition. Two other goats died (one goat died at 17 dpv, from the G4, and another goat at 38 dpv from the G2) before experimental challenge. All deaths were determined to be due to injuries or pre-existing conditions.

Goats in the G5 (unvaccinated control) group had higher median clinical scores relative to all other treatment groups (Table 4.1). Goats in all vaccinated (G1, G2, G3 and G4) had lower body temperature following virus challenge compared to the G5 (unvaccinated) controls ($P<0.001$).

In the G4, five goats developed muco-purulent nasal discharges between days 3 and 8 post-challenge. Four of the eight goats developed secondary lesions within 6-8 days post-challenge (Table 4.2). However, one goat never developed a secondary lesion or fever throughout the 14-day observation period.

Seven goats were challenged within the G3. None of the challenged animals in this group developed fever within the first 48 hours of challenge but by 72 h two goats had temperatures ≥40°C. In this group, one goat developed hyper-salivation at 3-day post-challenge without an obvious lesion at the site of inoculation or elsewhere on the oral mucosa. However, the oral swab from this goat tested positive for viral RNA at 4 days post-challenge and had a swelling at the site of inoculation on day 9. One other goat developed a secondary lesion at 8 days post-challenge

(Table 4.2).

In the G2, one goat did not develop lesions at the site of inoculation until day 5 post-challenge. The same goat developed interdigital lesions on both the left front and the right hind limbs at 7 days post-challenge (Table 4.2). One goat never developed any lesion at the site of inoculation or signs of fever throughout the 14-day observation period.

In the G1 (full dose vaccine) group, four goats developed lesions at the site of inoculation within 48 h post-challenge, while one goat developed lesions at the site of inoculation 72 h post-challenge (Table 4.2). However, none of the goats developed secondary lesions. The level of protection provided against development of clinical disease appeared to be dose-dependent (Supplemental Figure 4.2).

Table 4.1 Clinical onset of disease and median clinical scores of goats following intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool.

Group	Vaccine dose[a]	Day of challenge (dpv)[b]	Number of animals	Onset of disease at (dpc)	Median (Min – Max)* clinical score
1	Full dose (2 ml)	41	5	2-3	1 (0 – 4)[a]
2	1/3 dose (0.67 ml)	41	9	2-8	1 (0 – 5)[a]
3	1/6 dose (0.33 ml)	41	7	2-8	0 (0 – 2)[a]
4	1/12 dose (0.16 ml)	41	8	2-8	1 (0 – 10)[a]
5	Unvaccinated control (Placebo)[c]	41	5	2-7	12 (5 – 18)[b]

[a] The vaccine preparation used was the same for all the treatment groups. Different volume of the same vaccine concentration were used to adjust for doses. Animals were vaccinated by intramuscular route at one site in the neck. [b] Related to days of primary vaccination (days post vaccination = dpv). [c] The Unvaccinated Control (UVC) group was administered 2 ml of adjuvant placebo in the same order of the vaccination. Overall, there was a significant variation in the levels of clinical score across groups with the unvaccinated control group having significantly higher scores relative to the four vaccine groups ($P = 0.009$). *Overall significance $P<0.001$, Superscripts not in common denotes a significant difference ($P<0.05$).

Table 4.2 Summary of the clinical outcome in goats after intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool.

Days post challenge		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Groups	ID														
Group 1	L1	-	♦	-	-	-	-	-	-	-	-	-	-	-	-
(Full cattle dose)	L4	-	†	♦	-	-	-	-	-	-	-	-	-	-	-
	L9	-	♦†	-	-	-	†	-	-	†	-	-	-	†	-
	L19	†	♦	-	-	-	-	-	-	-	-	-	-	-	-
	137	†	♦†	-	-	-	-	-	-	-	-	-	-	-	-
Group 2	L5	-	-	-	-	♦	-	■	■	-	-	-	-	-	-
(1/3 cattle dose)	L15	†	-	♦	†	-	-	-	-	-	-	-	-	-	-
	L18	-	♦	-	-	-	-	-	-	-	-	-	-	-	-
	L20	-	♦	-	-	-	-	-	-	-	-	-	-	-	-
	L29	†	†	♦†	-	-	-	-	-	-	-	-	-	-	-
	146	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	156	-	†	♦	-	-	-	-	-	-	-	-	-	-	-
	162	-		♦	-	-	-	-	-	-	-	-	-	-	-
	160	-	†	♦	-	-	-	-	-	-	-	-	-	-	-
Group 3	L8	-	-		-	-	-	-	-	♦	-	-	-	-	-
(1/6 cattle dose)	L11	-	-	♦	-	-	-	-	-	-	-	-	-	-	-
	L12	-	-	†	♦	-	-	-	-	-	-	-	-	-	-
	L16	-	-	-	-	-	-	-	■	-	-	-	-	-	-
	L23	-	♦	-	-	-	-	-	-	-	-	-	-	-	-
	132	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	138	-	-	†	-	-	-	-	-	-	-	†	-	-	-
Group 4	L2	-	♦†	-	-	-	-	-	-	-	-	-	-	-	-
(1/12 cattle dose)	L14	-	♦	-	-	-		-	†	-	-	-	-	-	-
	L21	-	♦	-	-	-	■	-	-	-	-	-	-	-	-
	L22	-	♦†	†	-	-	-	■	†	-	-	-	-	-	-
	L27	-	♦	†	-	-	-	-	■	-	-	-	-	-	-
	140	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	157	-	-	♦†	-	-	-	-	-	-	-	-	-	-	-
	158	†	♦†	†	†	†	-	-	■	-	-	-	-	-	-
Group 5	L7	†	♦†	†	†	†	■	-	†	-	-	-	-	-	-
(Placebo)	L10	†	♦†	†	†	†	-	-	-	-	-	-	-	-	-
	L17	-	♦†	†	†	-	-	-	†	†	-	-	-	-	-
	L26	†	♦†	†	†	†	†	†	■†	†	†	-	-	-	-
	166	†	-	†	♦†	-	-	■	†	†	†	†	†	-	†

♦ = Lesion at the site of inoculation, ■ = Lesion at any other side including feet, mouth and tongue; indicative of generalized disease, † = Temperature ≥40°C.

4.3.3 Serological responses

All five goats in the G1 (full dose), 7/9 goats in the G2 and 6/7 goats in G3 had seroconverted by 20 days post-vaccination with mean PI antibody levels reaching a peak of 65%, 55% and 53% for the three groups respectively (Figure 4.1). The serological differences among groups were not different statistically ($P = 0.252$). The peak average PI occurred at 27 days post-vaccination for all four vaccine treatment groups, with the G1 (full dose), G2 and G3 reaching 86%, 84% and 83% mean percentage inhibition respectively. At seven days post-challenge, the mean antibody PI in all vaccinated groups had increased to >80% ($P<0.001$; Supplemental Table 4.1). There was a strong positive correlation between SPCE antibody levels and clinical protection (Spearman's $\rho = 1$; Figure 4.2). At study termination, all animals in the five treatment groups were seropositive to FMD SAT1 structural antibodies with mean antibody PI being highest for the full cattle dose group (Supplemental Table 4.1). All goats including the unvaccinated controls were positive for anti-3ABC non-structural protein antibodies at 7 days post-challenge (Table 4.3).

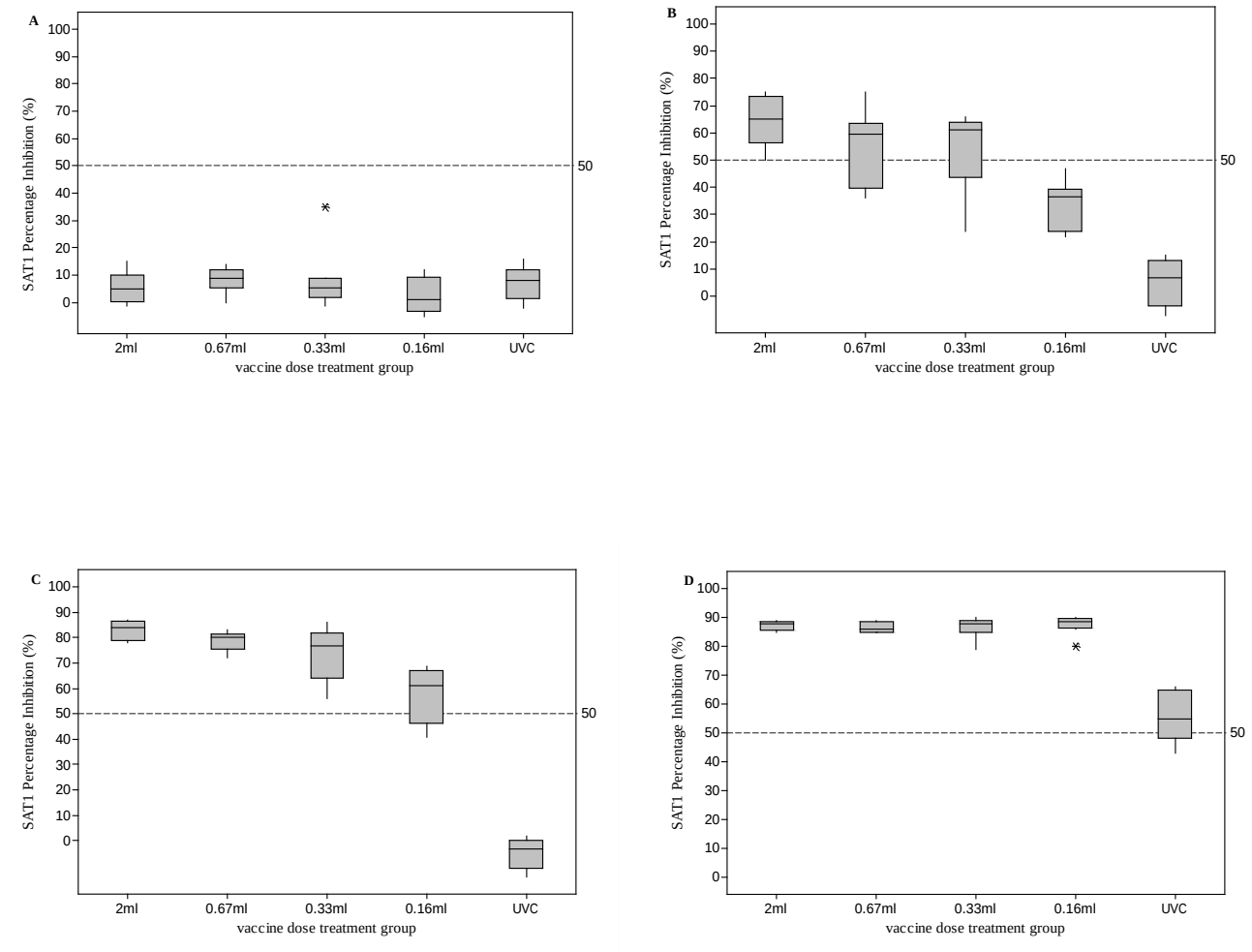


Figure 4.1 Descriptive presentation of SPCE FMDV SAT1 antibody levels across groups following vaccination showing median, first and third quartile values of antibody levels, the whiskers showing maximum and minimum levels and * showing outliers: (A) FMDV SAT1 pre-vaccination sera at day 0, (B) FMDV SAT1 antibody levels at 20 dpv, (C) FMDV SAT1 antibody levels at 41 dpv (challenge day), and (D) FMDV SAT1 antibody levels 48 dpv (7 dpc).

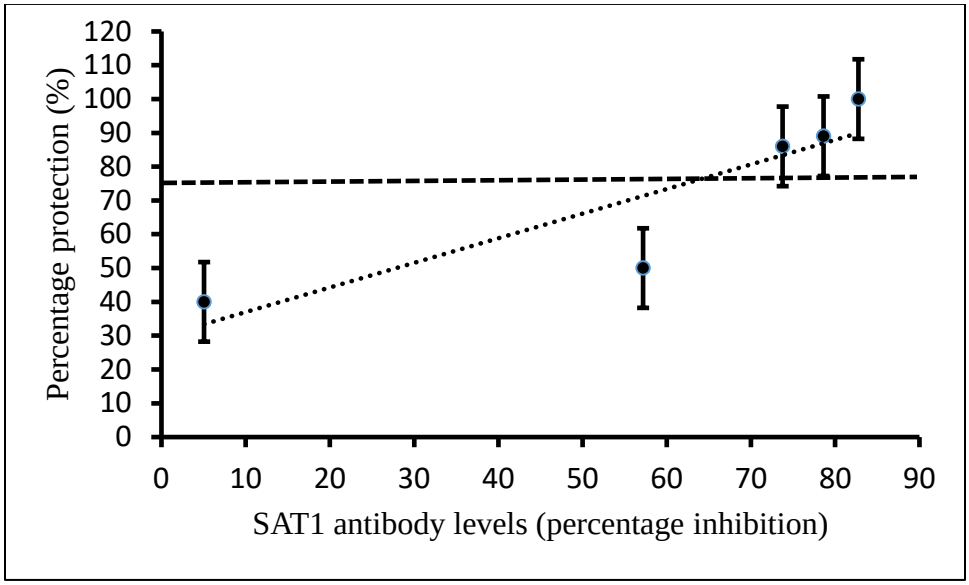


Figure 4.2 Correlation between SPCE antibody levels and clinical protection after intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool. Protection means animals that never developed secondary FMD lesions and a threshold of >75% is recommended for FMD vaccines (OIE, 2018). Data presented are at the vaccine treatment group level with error bars representing the 95% CI of the mean.

Table 4.3 Percentage protection of animals and 3ABC-specific antibody response following intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool.

Group	No. of animals Protected/challenged	Percentage Protection (95%CI)	3ABC ELISA (percentage reactors)	
			7 dpc (95%CI)	14 dpc (95%CI)
G 1 (Full cattle dose)	5/5	100 (55 – 100)	100 (55 – 100)	100 (55 – 100)
G 2 (1/3rd cattle dose)	8/9	89 (56 – 99)	100 (72 – 100)	100 (72 – 100)
G 3 (1/6th cattle dose)	6/7	86 (46 – 99)	100 (65 – 100)	100 (65 – 100)
G 4 (1/12th cattle dose)	4/8	50 (18 – 82)	100 (68 – 100)	100 (68 – 100)
G 5 (Placebo controls)	2/5	40 (7 – 82)	100 (55 – 100)	100 (55 – 100)

Protection means animals that had no secondary FMD lesions on the feet or away from the site of inoculation. dpc = days post challenge, CI = confidence interval.

4.3.4 Viral excretion

Three goats from the unvaccinated control group had viral RNA detected in nasal swab specimens at 2 days post-challenge, and by 6 days post-challenge all five goats had detectable viral genomic material (Table 4.4). Three goats from the G4 had viral RNA in the nasal swab at 6 days post-challenge. However, none of the goats from the G1 (full dose), G2 and G3 had evidence of viral RNA detected in the nasal swab specimens from 0-6 days post-challenge (Table 4.4). Viral RNA was detected from the oral swab of most goats from all five treatment groups by 48 h post-challenge and this extended until 6 days post-challenge for animals in the G4 and G5 (unvaccinated control) group (Table 4.4). However, none of the goats in the G2 had viral RNA detectable from oral swab specimens beyond 2 days post-challenge, and only one animal each from the G1 (full dose) and G3 had viral RNA detectable from oral swabs at 6 days post-challenge (Table 4.4).

Two goats from the G5 (unvaccinated control) group, and three goats from the G4 had viral RNA detectable in rectal swab specimens at 2 days post-challenge (Table 4.4). By 4 days post-challenge, all five goats in the G5 (unvaccinated control) group had detectable viral RNA in rectal swab specimens. Seven of the eight goats in the G4 had viral RNA detected in rectal swab specimens with one goat from the G3 at 4 days post-challenge. No viral RNA was detected from rectal swab specimens beyond 4 days post-challenge. There were significant effects of vaccine treatment group and specimen type on the magnitude of FMDV shedding (Table 4.5). Viral shedding of goats in the G4 did not differ significantly from the shedding of FMDV from the unvaccinated control goats ($P = 0.948$). However, there was a significant difference between the three larger vaccine dosages (G1, G2 and G3) and the other two groups ($P<0.001$). Goats in the G5 (unvaccinated control) group shed more virus compared to all vaccine groups except for the G4.

Table 4.4 Summary of the FMD viral RNA in clinical specimens from goats after intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool.

Days post challenge		0	2	4	6
Groups	ID				
Group 1	L1	-	O^+	O^+	O^+
(Full cattle dose)	L4	-	-	O^+	-
	L9	-	O^+	O^+	-
	L19	-	O^+	O^+	-
	137	-	O^+	-	-
Group 2	L5	-	-	-	-
(1/3 cattle dose)	L15	-	-	-	-
	L18	-	-	-	-
	L20	-	O^+	-	-
	L29	-	O^+	-	-
	146	-	O^+	-	-
	156	-	-	-	-
	162	-	-	-	-
	160	-	O^+	-	-
Group 3	L8	-	-	O^+	-
(1/6 cattle dose)	L11	-	O^+	R^+	-
	L12	-	-	O^+	-
	L16	-	O^+	O^+	-
	L23	-	O^+	O^+	O^+
	132	-	-	O^+	-
	138	-	-	O^+	-
Group 4	L2	-	O^+	$O^+ R^+$	O^+
(1/12 cattle dose)	L14	-	O^+	$O^+ R^+$	-
	L21	-	O^+	O^+	O^+
	L22	-	$O^+ R^+$	$O^+ R^+$	$N^+ O^+$
	L27	-	$O^+ R^+$	$O^+ R^+$	$N^+ O^+$
	140	-	-	$O^+ R^+$	$N^+ O^+$
	157	-	-	$O^+ R^+$	O^+
	158	-	$O^+ R^+$	$O^+ R^+$	O^+
Group 5 (Placebo)	L7	-	$N^+ O^+ R^+$	$O^+ R^+$	$N^+ O^+$
	L10	-	$N^+ O^+$	$N^+ O^+ R^+$	$N^+ O^+$
	L17	-	O^+	$O^+ R^+$	$N^+ O^+$
	L26	-	O^+	$N^+ O^+ R^+$	$N^+ O^+$
	166	-	$N^+ R^+$	$O^+ R^+$	$N^+ O^+$

N^+ = Viral RNA detected in nasal swab specimen, O^+ = Viral RNA detected in oral swab specimen, R^+ = Viral RNA detected in rectal swab specimen.

Table 4.5 Multivariable model estimates of fixed effects of vaccine treatment group for goats following intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool on the quantity of virus recovered from nasal, rectal and oral swabs.

Variable	Estimate (95%CI)	t statistics	P value
Experimental Group			<0.001
Group 1 (2 ml)	1.639 (0.783; 2.494)	3.789	<0.001
Group 2 (0.67 ml)	2.089 (1.334; 2.845)	5.469	<0.001
Group 3 (0.33 ml)	1.728 (0.934; 2.522)	4.306	<0.001
Group 4 (0.16 ml)	0.025 (-0.747; 0.183)	0.066	0.948
Group 5 (unvaccinated placebo)	Referent		
Specimen			
Nasal swab	2.398 (1.875; 2.922)	9.013	<0.001
Rectal swab	2.239 (1.718; 2.761)	8.458	<0.001
Oral swab	Referent		

CI = confidence interval, Ct-value was analysed and the lower Ct-value indicates more virus. Overall test for a difference among all treatment groups. Other P-values represent the comparison of individual groups to the referent.

4.4 Discussion

Vaccination is an important tool for the prophylactic control of FMD in endemic settings, where the goal is to reduce clinical disease and economic losses in livestock rather than eradication (FAO, 2016). The efficacy of a vaccine can be determined by using animal challenge studies based on reduction of clinical disease manifestation and viral shedding as detected by either virus isolation in cell culture or viral RNA on RT-PCR. It is recommended that the efficacy of FMD vaccines should be >75% compared to the unvaccinated control group based on protection against podal generalization (OIE, 2018). The aim of this study was to determine the efficacy of an experimental double-oil emulsion high potency FMD vaccine against disease and viral shedding in vaccinated and challenged goats. To our knowledge, this is the first study to evaluate the efficacy of a SAT serotype FMD vaccine in goats. Vaccination induced SAT1 antigen-specific immune responses as early as 14 days post-vaccination, with antibody levels for the three vaccine groups (G1, G2 and G3) reaching the antibody threshold level by 20 days post-vaccination. Vaccination also provided dose-dependent clinical protection among vaccinated groups with fewer secondary FMD lesions and reduced viral shedding compared to the unvaccinated group. This is similar to the results from a heterologous challenge study after vaccination with a high potency vaccine and challenge with serotype Asia-1 FMDV. In the previous study, vaccination reduced excretion of virus in nasal and oral secretions of sheep following intra-nasopharyngeal challenge (Horsington et al., 2018).

In the current study, there was a high proportion of sero-conversion in three of the vaccinated groups (G1, G2 and G3) by 20 days post-vaccination just prior to revaccination. Peak mean antibody levels of 86%, 84%, 83% and 77% occurred at 27 days post-vaccination in all four vaccine treatment groups respectively. This finding is consistent with a previous study using an oil-adjuvant vaccine in goats (Patil et al., 2002). A similar study in sheep also described higher

FMDV Asia-1 specific antibody levels by SPCE as early as 21 days post vaccination with a high potency vaccine (Horsington et al., 2018). The earliest time point for the detection of anti-FMD antibodies above 50% PI cut-off level after vaccination of goats with a high-potency vaccine (>6PD_{50}) is approximately 14 days (personal communication). This is at least 4-6 days later compared to cattle vaccinated with a similar high-potency vaccine. Furthermore, vaccination of goats with standard vaccine (3PD_{50}) may be further delayed. This observation in goats has been described to be common and might be due to the effect of adjuvant or differences in immune response between small and large ruminants. In the present study, all vaccinated groups had mean SPCE antibody levels above the positive threshold at the time of challenge, while all unvaccinated goats remained sero-negative. High potency FMD vaccines are known to induce rapid immune responses in sheep (Barnett and Carabin, 2002). In our study, there was a strong positive correlation between serological responses and clinical protection following challenge. Clinical protection against FMD has been previously reported to be associated in part with the induction of a serum antibody responses in sheep (Cox et al., 1999).

Non-structural protein antibody responses were detected in both vaccinated and unvaccinated animals as early as 7 days post-challenge. This is not a surprise for the vaccinated animals, where a rapid anamnestic response is expected, and the employed vaccine might not be highly purified and completely NSP-free. However, the appearance of NSP antibodies in the unvaccinated controls 7 days post-challenge is sooner than the 10-35 day range previously published for experimental infections in goats (Madhanmohan et al., 2011). The early anti-NSP response could thereforebe due to high levels of FMDV replication both in the vaccinated and unvaccinated goats. Although, in a study to evaluate the performance of a SAT serotype-specific 3ABC assay using specimens from cattle, three NSP assays (PrioCHECK®-NSP, IZSLER-NSP and SAT-NSP)

detected NSP antibodies at 5-7 days post-infection with SAT1 or SAT3 viruses with the exception of SAT1/NIG/5/81 infected animals which later tested positive at 14 days post-infection (Chitray et al., 2018). Antibodies to NSP have also been detected as early as 7-10 days in pigs following a FMDV O Taiwan challenge (Eblé et al., 2004).

Goats in the G4 were not protected against disease relative to higher vaccine dosages (G2 and G3). However, goats in the G2 had good protection relative to the goats in the G3 and G4. Intriguingly, one goat infected with the ½ virus challenge dose in the G3 remained FMD viral RNA negative for all specimens and NSP free without any signs of FMD throughout the study period. However, natural transmission occurred in the two sentinels maintained in this group (Lazarus et al., 2019). These results suggest that vaccination in combination with the reduced challenge dose induced protective immunity in this goat.

High potency O_1 Manisa FMD vaccines reduce virus excretion following homologous challenge in goats compared to unvaccinated controls (Madhanmohan et al., 2012). In this study, there was low viral shedding from clinical specimens collected from three vaccine groups (G1, G2 and G3), which might be a result of the dampening effect of vaccination. There was no evidence of viral shedding from nasal epithelium of goats in the higher vaccination treatment groups (G1, G2 and G3) and only 3 goats in the G4 had viral RNA detected in nasal swab specimens at 6 days post-challenge. Furthermore, there was no evidence of viral RNA excreted in rectal swab specimens beyond 4 days post-challenge. Since the goats were infected by the intra-dermolingual route, detection of viral RNA in oral swab specimens should not be considered a strong indication of systemic viral shedding, even though this drastically reduced to only two goats in the G1 and G3 by 6 days post-challenge. The hyper-salivation observed at 3 days post-challenge in a goat within the G3 might be a result of viral replication in the mucosal tissues since the same goat tested

positive for viral RNA in an oral swab specimen collected at 4 days post-challenge. Swelling also appeared at the site of inoculation at 9 days post-challenge. Vaccination of goats with low antigen payloads of an oil-adjuvant O_1 Manisa vaccine followed by homologous challenge reduced virus replication in the oropharynx, shedding of virus in nasal secretions and reduced the amount of virus released into the environment (Madhanmohan et al., 2011). One-half cattle dose of a high potency vaccine induced protective immune responses in goats after a homologous FMDV O_1 Manisa direct in-contact challenge (Madhanmohan et al., 2010b). Our results suggest that vaccine doses less than the one-half cattle dose might be sufficient to reduce FMDV transmission in goats. This would be advantageous due to the reduction in cost when vaccinating large populations of animals in endemic settings.

In most FMD vaccine efficacy studies conducted in cattle and sheep, protection from clinical disease did not always coincide with prevention of localized, subclinical infection. FMDV has been previously detected within the oropharynx of 50% of vaccinated goats within the first 10 days post challenge (Madhanmohan et al., 2011). In this study, we observed less viral RNA from oropharyngeal specimens in the larger dose vaccinated groups (G1, G2 and G3), which might suggest the ability of the vaccine to either prevent or reduce virus replication at the site of primary infection (oropharynx). This could theoretically reduce the amount of infectious material released into the environment from sub-clinically infected goats.

It is usual practice to employ a homologous challenge virus in FMD vaccine efficacy studies (Vosloo et al., 2015); however, we employed a pool of heterologous SAT1 2010 FMD viruses recovered from different time points during a single outbreak in cattle. Therefore, protection observed with this vaccine might have been higher if a homologous virus challenge was employed to the SAT1 strains in the vaccine. However, the SAT1 viruses used as a pool of the field challenge

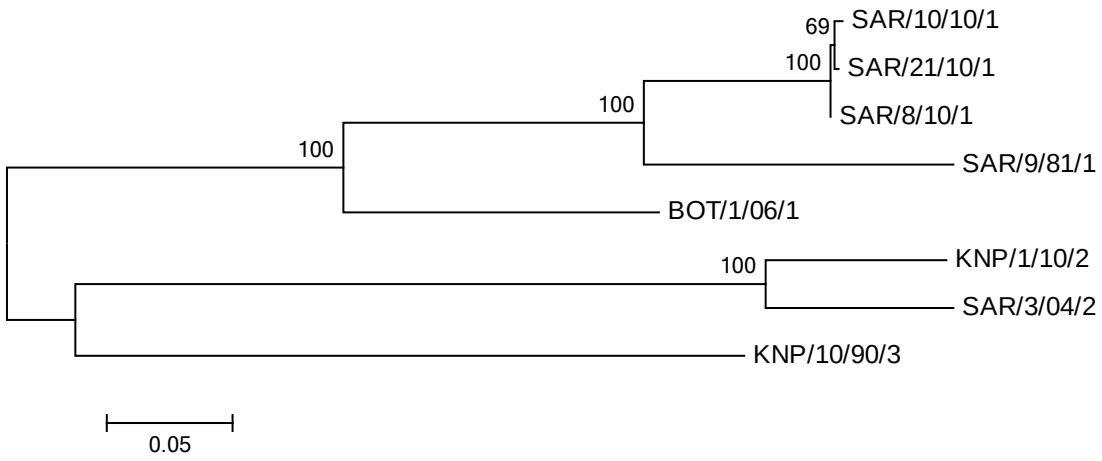
clustered closely to the SAT1 vaccine strains. Despite the close relationship, these findings demonstrate intra-serotype protection of the vaccine viruses against the challenge virus pool.

The results of this study should be evaluated in light of several limitations. The small number of animals allocated to each treatment group reduces the precision of our estimates. However, considering the principle of humane experimental technique employing the replacement, reduction and refinement concepts, working in a containment facility with animals requires minimum number for welfare reasons. In order to reduce the possibility of confounding, a stratified random allocation process was employed. Though, the limited number of goats available for the study in addition to the losses that occurred suggests that confounding still could have impacted reported results. Blinding was employed to limit information bias, especially in relationship to the clinical scoring. The different group sizes in conjunction with housing groups within independent animal rooms allowed blinded researchers to determine which two rooms housed the control groups versus the three rooms that contained the lower vaccine dosages. However, the development of primary FMD lesions at the site of experimental infection occurred within all treatment groups and it was therefore not possible for blinded researchers to determine individual group assignments. To improve the external generalizability of the results, animals of mixed sexes, body sizes and source were included in the study. Efficacy was determined based on the reduction of clinical disease and viral shedding only and it is a limitation that viraemia was not compared between groups. This study is also limited by the fact that we did not titrate the virus in goats to determine the optimal challenge dose prior to the current study. The paucity of clinical signs in some groups, especially the G3, suggests that the challenge system requires further refinement. Other limitations include the reliance on RT-PCR without virus isolation confirmation and not incorporating virus neutralization tests as an additional outcome to compare among treatment groups. Furthermore, it

would have been advantageous to include unchallenged sentinel goats in all treatment groups to determine the potential of vaccination to prevent natural transmission. Our inability to sequence-characterise the challenge virus before inoculation is another limitation to the study.

A fractional dose of one-third (1/3rd) the full cattle dose of a high potency double oil-emulsion FMD vaccine containing SAT1 virus strains can confer protection in goats against a heterologous challenge with a SAT1 FMDV pool. However, there is a need to further study the effect of the vaccine preparation on virus replication and duration of immunity after vaccination. Additionally, the evaluated vaccination schedule might not be feasible for use in endemic situations and further research is required to identify a cost-effective approach to vaccinating goats in southern Africa. It is also important to study other breeds of goats since exotic breeds are considered to be more susceptible to FMDV compared to animals that are indigenous to areas where FMD is endemic (Kitching, 2002). Presented information advances our knowledge of vaccine performance in goats, which should improve the progressive control of FMD in southern Africa.

SUPPLEMENTARY MATERIALS



Supplemental Figure 4.1. Neighbor-joining tree depicting partial VP1 sequences of SAT1, SAT2 and SAT3 foot-and-mouth disease viruses from southern Africa. The SAT1 challenge viruses (SAR/8/10/1, SAR/10/10/1 and SAR/21/10/1) cluster according to serotype. The type O virus from South Africa (SAR/19/2000) forms the outgroup. Bootstrap support values are shown near the nodes. Scale bar indicates 0.05 substitutions/site.

Supplemental Table 4.1 Peak antibody levels by SPCE Mean ± Standard Deviation PI (%) attained on the day of challenge (41dpv) and antibody responses following intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool from 48 to 55 days post-vaccination.

SAT1 Vaccine Group	dpv	Mean ± SD	P value*
Group 1 (2 ml)	41	82.75 ± 3.74	0.002
	48	87.20 ± 1.64	
	55	90.20 ± 1.92	
Group 2 (0.67 ml)	41	78.62 ± 3.61	<0.001
	48	86.77 ± 1.72	
	55	88.55 ± 2.78	
Group 3 (0.33 ml)	41	73.76 ± 10.24	<0.001
	48	86.50 ± 3.69	
	55	88.80 ± 5.25	
Group 4 (0.16 ml)	41	57.19 ± 10.87	<0.001
	48	88.50 ± 1.41	
	55	90.12 ± 1.25	
Group 5 (unvaccinated placebo)	41	-4.62 ± 6.52	<0.001
	48	56.20 ± 9.26	
	55	79.60 ± 4.22	

dpv = days post vaccination, SPCE = solid-phase competition ELISA, *Based on ANOVA test for a difference between sampling period.

CHAPTER FIVE

EXPLORING THE LIVESTOCK MOVEMENT NETWORK AMONG SMALLHOLDER GOAT FARMERS WITHIN THE FMD PROTECTION ZONE OF MPUMALANGA, SOUTH AFRICA

5.1 Introduction

Foot-and-mouth disease (FMD) is a highly infectious transboundary animal disease that affects cloven-hoofed livestock and wildlife including cattle, buffalo, pigs, sheep, goats, impala, deer and antelope (OIE, 2018; Poolkhet et al., 2019). The disease is caused by infection with foot-and-mouth disease virus (FMDV), a single-stranded RNA virus in the genus *Aphthovirus,* family *Picornaviridae* (Han et al., 2018). The virus spreads through the movement of infected and susceptible hosts (Brito et al., 2017; Tekleghiorghis et al., 2016). Effective movement controls, such as those implemented during the 2001 FMD epidemic in the United Kingdom, can slow down the spread of disease (Ferguson et al., 2001; Haydon et al., 2004). Goats are the most common livestock species kept by smallholder communal farmers in South Africa. However, the clinical signs of FMD in goats have been described to be mild and inapparent with nasal discharges, ulcerative lesions of the oral mucosa and the development of fever occurring in few animals (Lazarus et al., 2019). It has also been described that goats affected by FMD did not show more obvious sickness behaviors relative to cattle (Wolf et al., 2020). Goats can be thought to as “silent shedders” of disease because of their inability to present with obvious clinical signs of FMD as other cloven-hoofed animals. FMD is primarily transmitted through direct contact via inhalation (OIE, 2018; Poolkhet et al., 2019). Risk factors for the occurrence and spread of FMD include poor farm biosecurity practices (Ellis-Iversen et al., 2011; Megersa et al., 2009), presence of infected animals (Gibbens et al., 2001), presence of wildlife reservoirs (Molla et al., 2010; Vosloo et al., 1996), exposure to secretions or products derived from infected animals (Elnekave et al.,

2016), exposure to contaminated fomites (Alexandersen et al., 2003b), poor vaccination coverage (Jori et al., 2009; Nyaguthii et al., 2019), longer vaccination intervals (Lazarus et al., 2017), poor livestock inspection (Jori et al., 2009) and unvaccinated animal populations (Bravo de Rueda et al., 2014).

Livestock movement is one of the most important ways of spreading infectious diseases between holdings (Nremark et al., 2011). Many livestock diseases are transmitted through direct contact between animals, and thus between herds and flocks through animal movements. This has caused many countries of the developed world to register livestock movements using national databases (Anonymous, 2014). Information generated from such databases could be used for surveillance and planning disease control programmes. The rapid analysis of livestock movements could also be used to implement effective movement restrictions.

The application of social network analysis within the livestock industry has improved our knowledge of livestock movement patterns and has informed risk-based surveillance systems (Dubé et al., 2008; Poolkhet et al., 2019). Social network analysis has been used extensively to analyse livestock movements (Aznar et al., 2011; Green et al., 2008; Kao et al., 2006; Kiss et al., 2008; Mweu et al., 2013; Nremark et al., 2011; Robinson et al., 2007; Webb, 2006) and can identify targets for surveillance, intervention and control (Bajardi et al., 2012; Christley et al., 2005; Kiss et al., 2006; Natale et al., 2009). A network analysis study of the 2001 UK FMD outbreak (Shirley and Rushton, 2005), identified livestock markets and dealers as the hubs for disease spread. Certain nodes (some farms, animal markets and dealers) were key players in the early spread of FMD during the outbreak (Ortiz-Pelaez et al., 2006). All nodes had a high between-ness centrality, a measure of how frequent a node is located between each pair of node connections. Auction markets were key players for the spread of disease during this outbreak (Robinson and Christley, 2007). A

similar study in Denmark also reported that cattle markets influenced other nodes within the Danish cattle movement network (Mweu et al., 2013). The transportation of infected cattle is known to be responsible for disease transmission (Dubé et al., 2008) and the out-degree centrality, a quantification of the number of outgoing ties from the node, is useful for estimating the resulting size of the outbreak. The control of infectious livestock diseases such as FMD should focus on livestock movements within the cattle trade network (Natale et al., 2009).

The aim of this study was to understand the role that movement of livestock plays in the spread of disease within an FMD protection zone of South Africa with the need to identify high-risk locations for improved surveillance and strategic vaccination programme.

5.2 Materials and methods

5.2.1 Study area

This study was conducted in the Mnisi Tribal Authority (MTA), a communal farming area within the FMD Protection zone with vaccination in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa (Figure 5.1). The MTA is divided into three animal health wards (Bushbuckridge East, Animal Health wards I-III) and totals 16 communal dip tanks (livestock inspection points). Communal farmers within this area are involved mostly in livestock rearing. The proximity to the Kruger National Park (KNP) poses a threat to livestock production due to infectious diseases and the surrounding areas have poor market access due to situation within the FMD Protection zone with vaccination (Lazarus et al., 2018).

FMD control measures in South Africa include the separation of wildlife and livestock using fences, clinical surveillance, routine vaccination of cattle and movement control of susceptible livestock, wildlife and their products (DAFF 2014). According to the South African veterinary legislation, three zones exist for the control of FMD (DAFF 2014). The three zones classify the

country into: a) FMD Infected zone b) FMD Protection zone (with or without vaccination and c) formerly FMD Free zone (majority of the country). The protection zone protects the status of animals in the FMD Free zone and movement of livestock into the free zone is restricted using a permit system after the animal has been examined and certified to be free from FMD.

Farmers within the protection zones are mostly engaged in communal farming activities, which are considered to be a cost-effective farming system (Dovie et al., 2006). However, it is a high-risk husbandry system due to poor biosecurity practices that might lead to the occurrence and spread of diseases including FMD. As a control measure for FMD and other infectious diseases, movement of cattle out of this zone of the country is only allowed if the animal originates from a herd that has evidence of previous FMD vaccination and has a movement permit issued by the official veterinary service.

Between January and November 2019, two FMD serotype SAT2 outbreaks were reported in the formerly FMD Free zone of Limpopo Province, (DAFF 2019a; DAFF 2019b). These outbreaks caused South Africa to lose the World Organisation for Animal Health (OIE) certified Free zone status without vaccination.

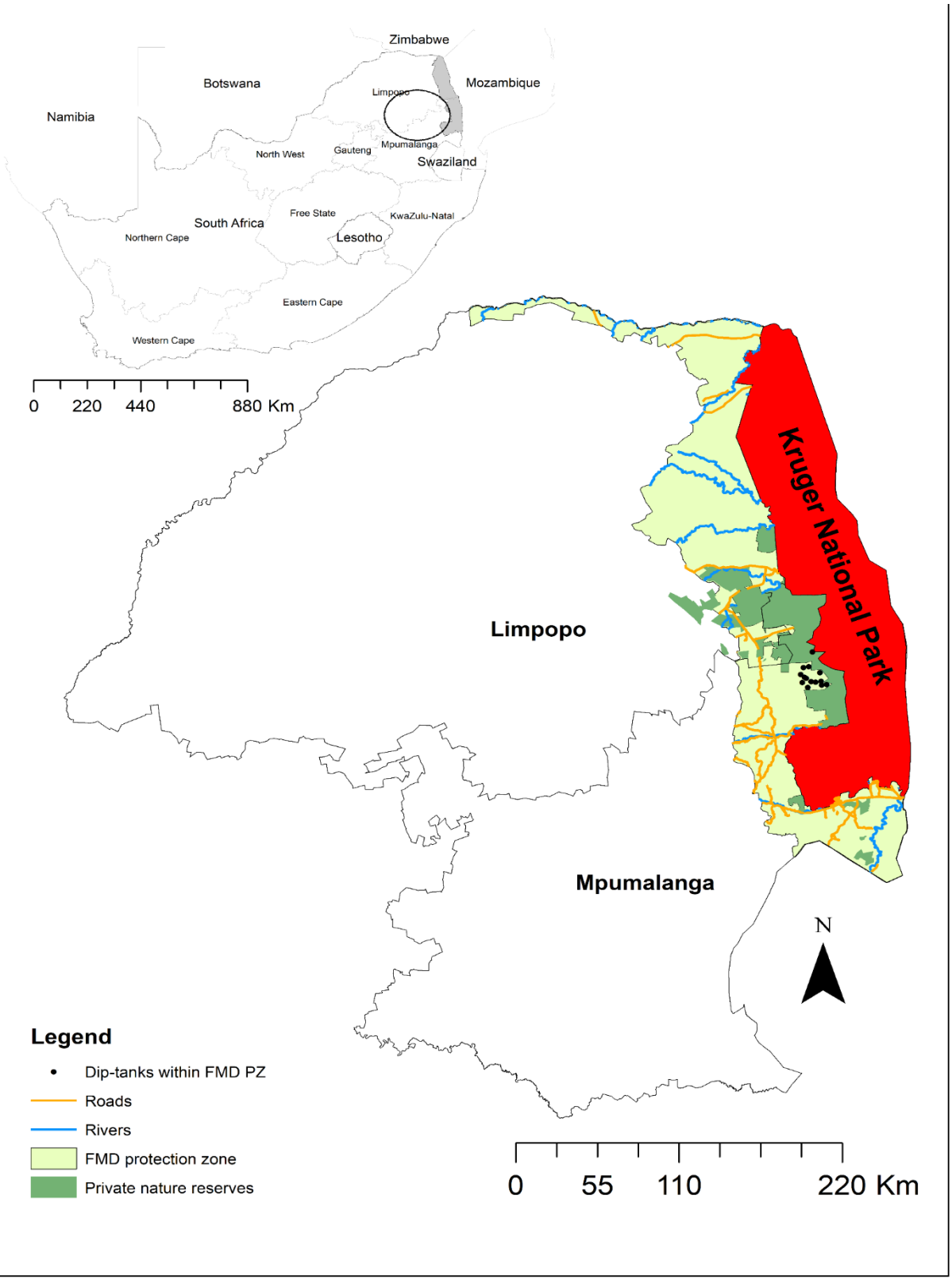


Figure 5.1 Map of the study area showing the distribution of communal dip tanks where the survey was conducted during June to July 2018.

5.2.2 Target population and sample size calculations

The target population was smallholder farmers who kept goats within the three animal health wards of the MTA. The required sample size was calculated based on the desire to estimate the proportion of respondents that reported small ruminant movement (in and out) of their flock at least once during a one-year period (July 2017 –June 2018). Given the lack of knowledge of small ruminant movements in the area, the proportion was assumed to be 50% and calculations were based on a desired confidence level of 95% and absolute error of ±10%. The sample size was estimated to be 97 respondents (Thrusfied, 2005) but increased by 5% to account for non-response and the possibility of data exclusions. Participants were selected proportional to the total number of registered livestock owners (stratified by small stock) per communal dip tank (Supplemental Table 5.1).

5.2.3 Ethical consideration

The study was approved by the University of Pretoria, Faculty of Veterinary Science, Animal Ethics Committee (Project Number V022-17) and the Faculty of Humanities, Ethics Committee (Project Number GW20170623HS). Participants were presented with a consent form before the commencement of interviews or focus group discussions and their identity was coded to ensure confidentiality. Verbal consent was obtained from illiterate respondents.

5.2.4 Data collection

5.2.4.1 Questionnaire development and administration

The questionnaire was pilot tested among 12 smallholder farmers from a community in Northwest Province, South Africa prior to finalization and administration in this study. The final questionnaire comprised a total of 21 questions divided into the following four sections: owner demographics, herd demographics, animal management and animal movement and losses. A

combination of open and closed questions was used. For some questions, respondents were asked to choose only the most applicable answer, while for others they could select all appropriate options.

A semi-structured interview was administered in Xitsonga language using a trained interviewer. Smallholder goat farmers were individually recruited on a voluntary basis as they appeared for the weekly livestock inspection at the dip tanks. Respondents were enrolled after being informed of the study purpose. The interview session lasted for approximately 30 minutes with each respondent. Interviews were conducted at the communal dip tanks, which is the usual meeting point for all farmers during the weekly livestock inspections. Global positioning system (GPS) coordinates for all study locations were captured using a handheld device (Garmin eTrex® 10, USA) at the time of the interview.

5.2.4.2 Focus group discussions and participatory mapping

For the focus group discussion and the participatory mapping, a semi-structured interview was conducted with separate groups at the communal dip tanks. Participants were recruited on a voluntary basis after being informed of the study purpose. Sessions were split into multiple groups with a maximum of 11 participants when group sizes were large. Respondents were requested to identify origins and destinations of livestock movement on a sketch map display of the MTA using laminated pictures of goats and cattle. Participants were further asked to list the origin and destinations of animal movements outside the three animal health wards. Questions were also asked concerning reasons for buying and selling animals and challenges faced in goat production. The local animal health technicians also participated in a group session to validate collected data and remove uncertain ties (origin and destination pairs) from the network.

5.2.4.3 Additional animal health information

Additional information concerning livestock distributions, inspection data and cattle vaccination coverage for the period preceding the study (2017/2018) was reviewed from the local animal health technician's records.

5.2.4.4 Network properties

The network of goat and cattle movements in the three animal health wards of the MTA were analysed using directed and symmetrized methods (Borgatti et al., 2013). Nodes were the communities (group of people living together and sharing a communal dip tank facility for animal health and handling) within the wards and the ties were live goat and cattle movements between communities. A descriptive statistical analysis was performed and network analysis was calculated on a directed binary network using UCINet6.66.4 (Analytical Technologies, USA) (Borgatti et al., 2013). The following indices were used for the calculation of the network centrality measures:

The degree centrality is the normalized value accounted for by analyzing the number of ties in each node. The node with a high value reflects a high number of ties or the channel of node connection. Directed networks are best described as either out-degree or in-degree centrality, based on whether the ties are directed away from a node or directed toward a recipient node.

The between-ness centrality is the Freeman normalized value, which is considered the shortest path between two nodes. The node with a high value indicated a high frequency of animal movements through the node.

The closeness centrality is a normalized value that is considered the geodesic distance from one node to all remaining nodes. A node with a high value indicated that it is easy to move animals to the linking node.

The clustering coefficient is calculated from three connected nodes forming a triangular shape (transitivity) in the network. A network with a high clustering coefficient means that many node triangles are present.

The network density is the proportion of actual ties that are present in the network out of all possible ties.

5.2.5 Data analysis

Descriptive statistics were presented as frequencies and percentages. Continuous data were described either using mean ± standard deviation or medians and interquartile ranges (IQR). The normality assumption for quantitative variables was assessed by calculating descriptive statistics, plotting histograms and performing the Anderson-Darling test for normality within MINITAB Statistical Software, Release 16 (Minitab Inc., USA). Normally distributed variables were presented as means ± SD and comparisons performed using one-way ANOVA. Kruskal-Wallis tests were used to compare centrality measures across the three animal health wards of the study area. The Wilcoxon signed-rank tests was used to compare centrality measures between goat and cattle movement networks. Statistical analyses were performed in commercially available software (IBM SPSS Statistics Version 24, International Business Machines Corp., Armonk, New York, USA) and significance was set at $P<0.05$. Mapping of the networks was performed using ArcGIS 10.2.3 (ESRI, USA). Nodes were projected using their GPS coordinates estimated using Google Earth (https://www.google.com/earth/) when outside the study area.

5.3 Results

5.3.1 Demographic and husbandry findings

A total of 116 smallholder goat farmers were interviewed during June-July 2018, with 36 respondents from Ward I, 35 respondents from Ward II, and 45 respondents from Ward III. The

median (IQR) age of respondents for the three wards was 62 (53 – 75), 65 (52 – 79) and 66 (55 – 74) for Wards I-III, respectively. The major occupation of respondents was livestock farming with some also involved in private or government employment (Table 5.1). In addition to rearing goats, some respondents also reared cattle, pigs and chickens (data not presented). The median (IQR) experience in farming was 27 (14 – 38), 28 (11 – 28) and 19 (10 – 27) years for Wards I-III, respectively. Eighty-three percent (30/36), 86% (30/35) and 80% (36/45) of respondents kept cattle in addition to goats in Wards I-III, respectively. Respondents from Ward I indicated that their motivation for farming included love for animals (31%), subsistence (22%), business (19%), draught power (6%), ceremonial and cultural purpose (31%) and long-term savings and investment (3%). For the respondents in Ward II, these included subsistence (51%), love for animals (20%), draught power (11%), business (9%) and long-term savings and investment (6%). Motivating factors for respondents in Ward III included subsistence (42%), business (22%), love for animals (13%), long terms savings and investment (11%), ceremonial and cultural purposes (7%) and draught power (2%).

Age of respondents, level of education, farming experience and number of goats owned were not different among the three animal health wards (Table 5.2). A total of 134 participants attended the focus group discussion from across the three animal health wards, with 68% (91/134) males and 32% (43/134) females.

Seventy-five percent (27/36) of respondents from Ward I indicated that they kept goats in a separate holding facility away from cattle, while 94% (33/35) respondents from ward II kept goats separate from cattle. However, in Ward III, all respondents, 100% (45/45) indicated they kept goats separate from cattle. Forty-seven percent (17/36) of respondents from Ward I, 26% (9/35) of respondents from Ward II and 27% (12/45) of the respondents from Ward III provided their

animals with supplemental feed in addition to the open grazing system. Most respondents obtained the supplemental feed from the Agricultural Cooperative Stores (data not presented). A total of 134 participants attended the focus group discussion from across the three animal health wards, with 68% (91/134) males and 32% (43/134) females.

Table 5.1 General demographic data of respondents for the three Bushbuckridge Animal Health wards in Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa during June 2018 (n=116).

Variable	Number of respondents (%)		
	Animal Health Ward 1 (n = 36)	Animal Health Ward 2 (n = 35)	Animal Health Ward 3 (n = 45)
Sex			
Male	24 (67)	28 (80)	28 (62)
Female	12 (33)	7 (20)	17 (38)
Age			
<20 years	0 (0)	1 (3)	0 (0)
20 – 40 years	6 (17)	1 (3)	4 (5)
41 – 60 years	12 (33)	14 (40)	21 (47)
61 – 80 years	10 (28)	7 (20)	13 (29)
>80 years	6 (17)	5 (14)	5 (11)
No response	1 (3)	7 (20)	2 (4)
Marital status			
Single	2 (6)	2 (6)	6 (13)
Married	25 (69)	21 (60)	25 (56)
Divorced	0 (0)	0 (0)	0 (0)
Widowed	9 (25)	12 (34)	14 (31)
Education			
Non formal	11 (31)	16 (46)	22 (48)
Primary school	5 (14)	5 (14)	11 (24)
Secondary school	15 (42)	11 (31)	9 (20)
Tertiary	0 (0)	0 (0)	1 (2)
No response	5 (14)	3 (9)	2 (4)
Main occupation			
Livestock	20 (56)	17 (49)	33 (73)
Crops	0 (0)	0 (0)	1 (2)
Government employee	1 (3)	3 (9)	3 (7)
Private sector	3 (8)	2 (6)	5 (11)
Own business	9 (25)	8 (23)	0 (0)
General employee	0 (0)	3 (9)	2 (4)
House keeping	1 (3)	1 (3)	0 (0)
No response	0 (0)	1 (3)	0 (0)
Goat rearing experience			
<10 years	5 (14)	6 (17)	8 (18)
10 – 20 years	5 (14)	4 (11)	12 (27)
21 – 30 years	5 (14)	4 (11)	7 (16)
>31 years	14 (39)	19 (54)	18 (40)

Table 5.2 The association between the three animal health wards with potential continuous predictors in 116 respondents sampled within the Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa during June 2018.

Variable	Ward I		Ward II		Ward III		
	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	P – value*
Age of respondents (years)	23	62 (51 – 80)	18	68 (66 – 70)	24	65 (56 – 71)	0.371
Level of education	19	7 (0 – 12)	14	4 (0 – 11)	24	2 (0 – 6)	0.135
Farming experience (years)	14	31 (18 – 54)	13	27 (16 – 39)	16	20 (13 – 28)	0.198
Number of goats owned	23	11 (6 – 15)	18	8 (6 – 13)	24	10 (5 -12)	0.591

IQR = Interquartile range. *Based on Kruskal-Wallis tests comparing variables among the three animal health wards. Age of respondents H statistic is 1.985 (2, N = 65), Level of education H statistic is 4.000 (2, N = 57), Farming experience H statistic is 3.236 (2, N = 43) and Number of goats owned by respondents H statistic is 1.047 (2, N = 65).

5.3.2 Animal movements

There were less reported movements into holdings based on questionnaire responses relative to movement out of the holdings during the previous 12 months (Table 5.3). Reported movement into holdings were 5 goats in Ward I, 17 goats in Ward II and 11 goats in Ward III. The corresponding number of movements for cattle was 12 heads of cattle in Ward 1, 6 heads of cattle in Ward II, and 5 heads of cattle in Ward III. Respondents from Ward I indicated the most recent time of animal movement into the holdings to be median 6 (1 – 30) months for goats and median 2 (1 – 7) months for cattle. The most recent time for animal movement into the holdings for respondents in Ward II were median 6 (4 – 12) months for goats and median 8 (3 – 12) months for cattle. Respondents in Ward III indicated a median time of median 5 (3 – 12) months for goats and only 2 months for cattle. Livestock movement out of the flock for the previous 12 months preceding the study was 45 goats and 30 heads of cattle in Ward I, 38 goats and 25 heads of cattle in Ward II and 36 goats and 35 heads of cattle in Ward III. The most recent reported time of animal movement out of the holdings were median 6 (2 – 11) months for goats and median 2 (1 – 7) months for cattle in Ward I, median 2 (1 – 8) months for goats and median 3 (2 – 9) months for cattle in Ward II, and median 4 (1 – 6) months for goats and median 3 (2 – 8) months for cattle in Ward III. Almost half of the respondents reported losses of livestock with descriptively more goats lost during the period relative to cattle (Table 5.3).

Table 5.3 Responses of respondents to questions towards animal management, animal movements and animal losses within the three animal health wards of Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa during June 2018.

Variable	Ward I (n = 36) Frequency		Ward II (n = 35) Frequency		Ward III (n = 45) Frequency	
	n	%	n	%	n	%
Livestock movement "into" the previous 12 months						
Yes	9	25	9	26	8	18
No	27	75	25	71	36	80
Reasons for moving in animals						
Farming	6	17	6	17	5	11
Business	0	0	3	9	0	0
Rituals	0	0	0	0	1	2
Gift	1	3	0	0	1	2
Inheritance	1	3	0	0	1	2
Separating new animals from the herd/flock						
Yes	6	17	9	26	2	4
No	26	72	23	66	38	84
Livestock movement "out" of holdings in the previous 12 months						
Yes	23	64	18	51	24	53
No	12	33	17	49	21	47
Reasons for moving and selling animals						
Emergency need of funds	3	8	0	0	0	0
Daily expenses	2	6	3	9	0	0
Consumption	4	11	4	11	6	13
Injury/illness	0	0	1	3	0	0
Culling	2	6	1	3	3	7
Pay medical bills	2	6	5	14	10	22
Pay school fees	2	6	4	11	4	9
Rituals/social events	7	19	0	0	1	2
School fees/medical bills	1	3	0	0	0	0
Requirement for permit to move goats from holdings						
Yes	13	36	5	14	6	13
No	23	63	26	74	21	47
Requirement for permit to move pigs from holdings						
Yes	7	19	1	3	2	4
No	8	22	9	26	7	16
Requirement for permit to move chickens from holdings						
Yes	8	22	0	0	0	0

No	18	50	14	40	13	29
Selling sick goats from the flock						
Yes	7	19	1	3	0	0
No	29	81	31	86	44	98
Livestock sales (goats)						
Butchers	0	0	0	0	3	7
Middlemen	33	92	31	89	36	80
Auction	1	3	0	0	2	4
Traditional healers	1	3	1	3	3	7
Middlemen/traditional healers	1	3	0	0	0	0
Animal losses in the previous 12 months						
Yes	16	44	12	34	21	47
No	20	56	21	60	24	53
Species						
Cattle	16		9		17	
Goats	56		30		90	
Reasons for losses						
Stock theft	5	14	3	9	9	20
Illness/death	3	8	1	3	2	4
Killed by dogs	7	19	5	14	4	9
Declared missing	1	3	1	3	1	2

5.3.3 Network analysis of goat movements

Data from 116 questionnaires and 13 focused group discussions reported 37 nodes (communities) and 78 ties (links between respective communities) with an overall network density of 0.059 (SD = 0.235) across the study area. Most of the nodes had connections with each other, with extension to nodes outside the study area (Figure 5.2). Clare B in Ward I and Eglington in Ward II had the largest in-degree centrality values of 9 and 7 respectively. Moreover, the first five nodes with the highest values for out-degree centrality were Eglington (12), Share (11), Tlhavekisa (7), Shorty (7), and Athol (7). Ten nodes had links with communities outside the study area and animals were routinely moved to these outside communities either for consumption or husbandry purposes. On average, the actors (respective nodes of the study network) had a degree of 2.11 for both in-degree and out-degree, which was quite low given that there were 37 actors in the network. Overall, the network had 16 nodes within the study area and 21 nodes outside the study area. Four locations within the FMD free zone of the country (Nelspruit, Tzaneen, Barbertone and Leboeng) had links with the movement of goats from the study area. The range of out-degree was slightly higher (minimum – maximum: 0 – 12) than that of the in-degree (0 – 9) with more variability across actors in the out-degree than the in-degree (standard deviation and variance). The network had an out-degree coefficient of variation of 154 and in-degree coefficient of variation of 89. In this network, the out-degree graph centralization was 28% and the in-degree graph centralization was 20% of the theoretical maximums.

Closeness centrality measures indicated Eglington and Share, both in Ward II to be the nodes with the highest out-degree closeness values followed by Seville B in Ward III and Tlhavekisa in Ward I. There was more variation in the out-closeness value relative to the in-closeness (minimum – maximum: 0 – 20.33).

Eglington and Shorty both in Ward II, Tlhavekisa (Ward I) and Hlalakahle (Ward III) had the largest between-ness measures. There was a lot of variation in actor between-ness (range 0 – 175), and that there was a wide variation (standard deviation = 37.34 relative to a mean between-ness value of 17.18). Despite this, the overall network centralization was relatively low (12.91%) with an overall graph clustering coefficient of 0.248.

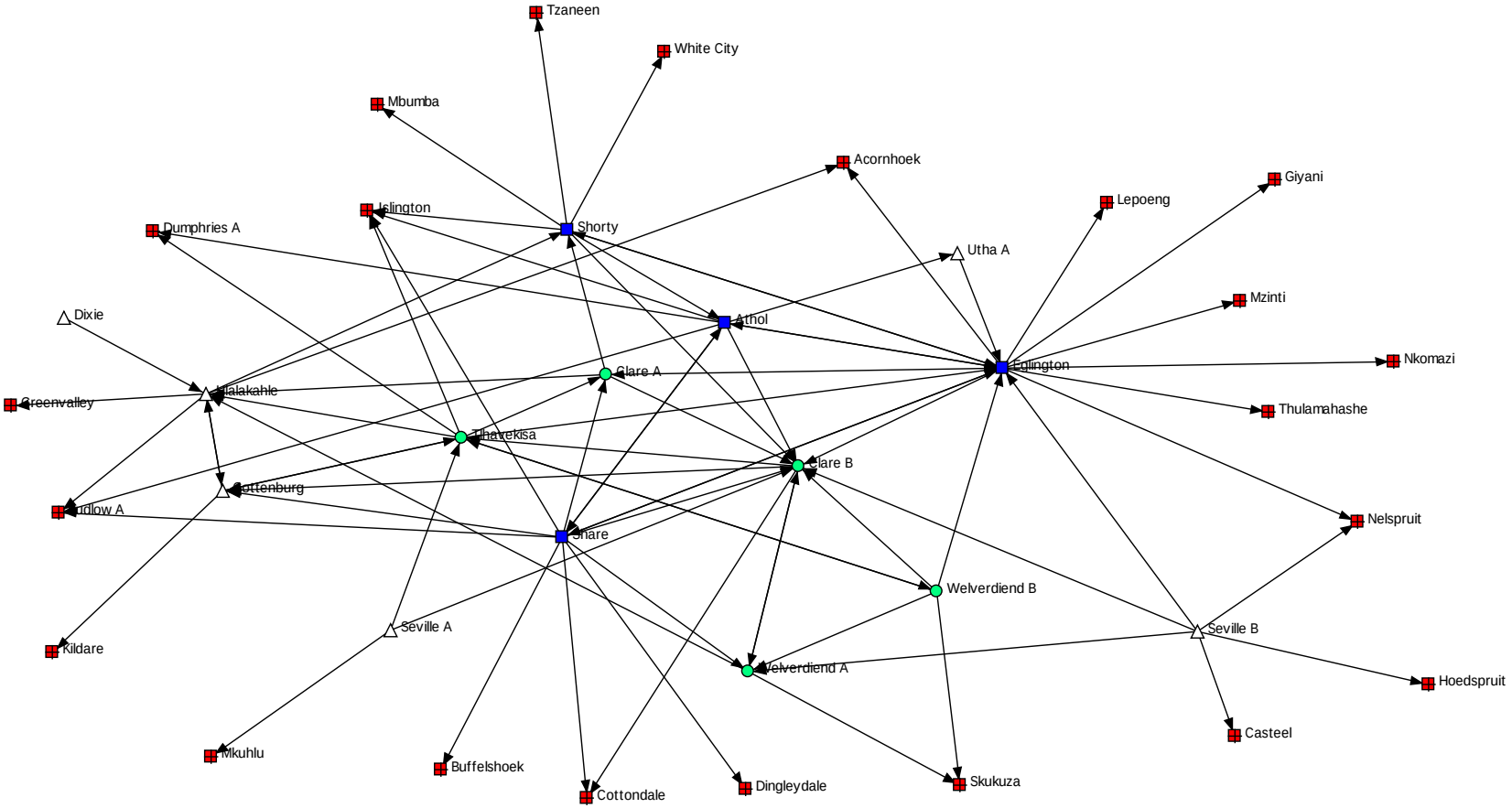


Figure 5.2 Goat movement patterns in three animal health wards of Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa. Node shapes and colours indicate locations; green circle = Ward I, blue box = Ward II, colourless triangle = Ward III and red boxes are nodes outside of the study area.

5.3.4 Network analysis of cattle movements

Overall, data from 116 questionnaires and 13 focused group discussions represented 42 nodes (communities) and 90 ties (links between respective communities) with an overall network density of 0.052 (SD = 0.223). Most of the nodes had connections with each other, with extension to nodes outside the study area (Figure 5.3). Gottenburg and Seville B both in Ward III, had the highest out-degree centrality values of 10 and 9, respectively. Giyani, a node in Limpopo Province and Thulamahashe, a node in Mpumalanga Province both had higher in-degree centrality measures of 12 and 8 respectively. Thirteen of the nodes (87%) had links with communities outside of the study area. On average, the actors had a degree of 2.14 for both in-degree and out-degree, which was quite low given that there were 42 actors in the network. Overall, the network had 16 nodes within the study area and 26 nodes outside the study area. The range of in-degree was slightly higher (minimum – maximum: 0 – 12) than that of the out-degree (0 – 10) and there was little variability across actors in-degree and out-degree (in-degree mean = 2.14, out-degree mean = 2.14, in-degree SD = 2.27, and in-degree variance = 7.26, out-degree variance = 7.41). The network had an out-degree coefficient of variation of 127 and an in-degree coefficient of variation of 125. The out-degree graph centralization was 20% and the in-degree graph centralization was 25% of the theoretical maximums.

Gottenburg and Seville B (both in Ward III), and Tlhavekisa and Clare B (both in Ward I) were the nodes with the highest out-degree closeness values. There was little difference between the in-closeness and out-closeness values for the network.

Clare B (Ward I), Athol and Eglington (both in Ward II) had larger between-ness measures compared to other dip tanks in the network. However, there was a large variation in actor between-ness (from 0 – 217) relative to a mean between-ness value of 31.12. Despite this, the overall

network centralization index was relatively low (12%) and the network had an overall graph clustering coefficient of 0.129.

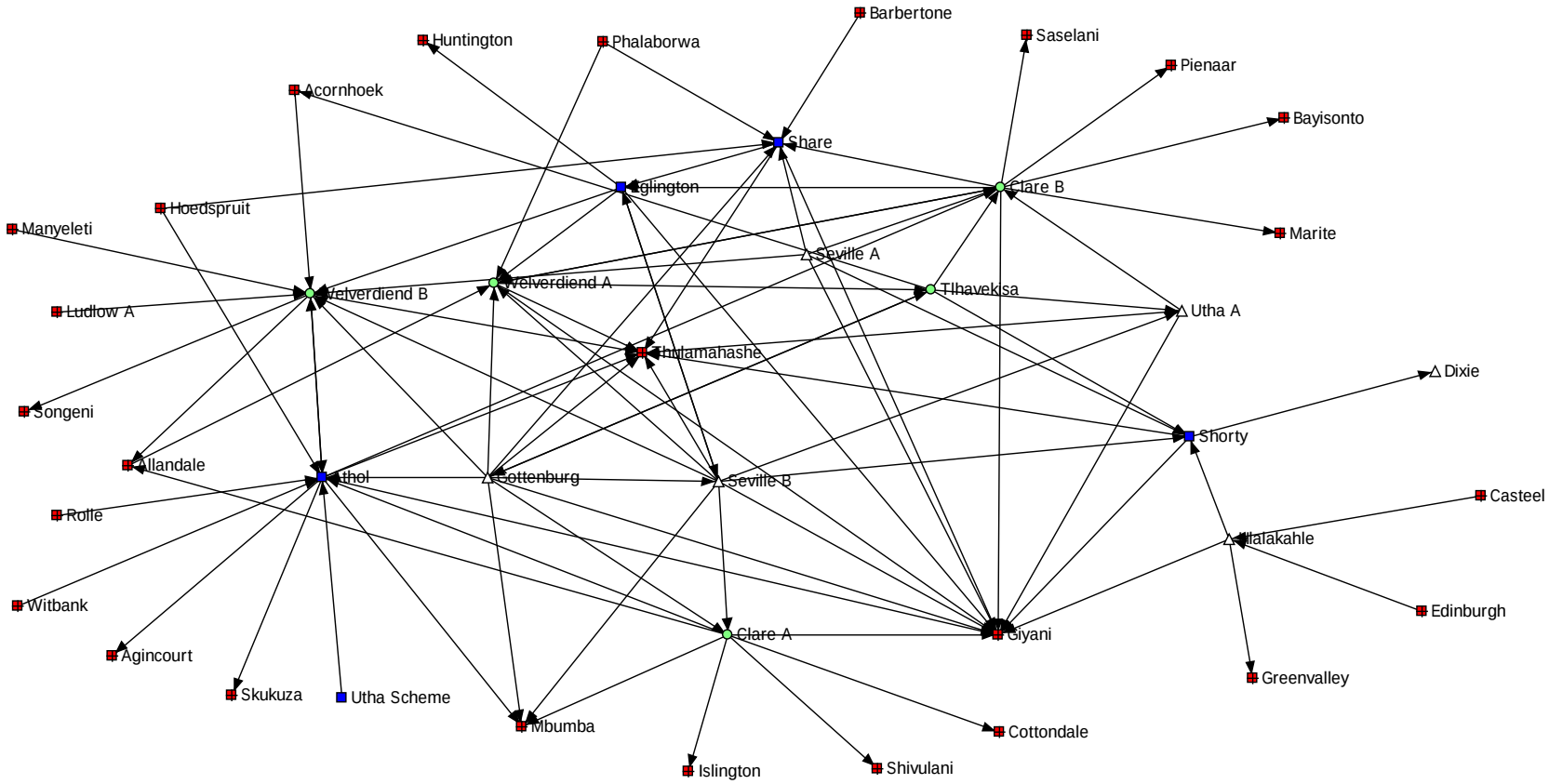


Figure 5.3 Cattle movement patterns in three animal health wards of Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa. Node shapes and colours indicate locations; green circle = Ward I, blue box = Ward II, colourless triangle = Ward III and red square boxes are nodes outside of the study area.

5.3.5 Comparison of movement network centrality measures

There were no significant differences of movement centrality measures among areas (Tables 5.4 and 5.5). However, median out closeness and between-ness centrality measures for the network were significant by species, with goat movement network having more out-closeness centrality (P = 0.021) and cattle network having more between-ness centrality (P = 0.008; Table 5.6).

5.3.6 Additional animal health information and FMD history

The number of goats and the inspection efficiency descriptively varied by dip tank. However, there was no significant difference in goat numbers among wards (P = 0.277). The number of pigs in the study area was small and there was 100% inspection efficacy. Animal health Wards I and III, had fewer pigs relative to Ward II (P<0.001). Cattle FMD vaccination coverage for the period preceding the study descriptively varied by dip tank (Table 5.7). The number of cattle within the three wards was not significantly different (P = 0.306). Cattle from Wards I and II had a longer inter-vaccination interval (>220 days) than the 120-day inter-vaccination interval prescribed by South African veterinary services. During 2017, a SAT2 FMD outbreak was reported at Tlhavekisa, a community within the animal health Ward I (OIE-WAHID, 2017). However, the outbreak was contained within the community without spreading to other dip tanks within the study area.

Table 5.4 Goat movement network centrality measures in the three animal health wards of Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa during June 2018.

Variable	Animal Health Ward			
	Ward I Median (IQR)	Ward II Median (IQR)	Ward III Median (IQR)	P – value*
In-degree	4.00 (2.0 – 6.50)	3.00 (2.25 – 6.00)	0.50 (0.00 – 4.25)	0.612
Normalised In-degree	11.11 (5.55 – 18.06)	8.33 (6.25 – 16.66)	1.39 (0.00 – 11.81)	0.547
Out-degree	4.00 (3.00 – 6.00)	9.00 (7.00 – 11.75)	3.00 (1.00 – 5.25)	0.513
Normalised Out-degree	11.00 (3.00 – 6.00)	25.00 (19.44 – 32.64)	8.33 (2.78 – 14.59)	0.513
InClosesness	8.25 (6.79 – 9.92)	7.92 (6.92 – 9.67)	0.00 (0.00 – 8.66)	0.579
Normalised InClosessness	22.92 (18.86 – 27.55)	21.98 (19.21 – 26.85)	0.00 (0.00 – 24.77)	0.579
OutCloseness	14.00 (12.86 – 16.66)	18.46 (17.12 – 20.16)	13.67 (11.75 – 16.04)	0.699
Normalised OutCloseness	38.89 (35.74 – 46.30)	51.28 (47.57 – 56.02)	37.96 (32.66 – 44)	0.699
Between-ness	16.90 (9.40 – 74.90)	70.30 (32.10 – 156.80)	0.00 (0.00 – 52.40)	0.346
Normalised Between-ness	1.34 (0.75 – 5.95)	5.58 (2.45 – 12.44)	0.00 (0.00 – 4.16)	0.346

*Based on Kruskal-Wallis tests comparing variables among the three animal health wards. IQR – Interquartile range.

Table 5.5 Cattle movement network centrality measures in the three animal health wards of Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa during June 2018.

Variable	Animal Health Ward			
	Ward I Median (IQR)	Ward II Median (IQR)	Ward III Median (IQR)	P – value*
In-degree	5.00 (2.00 – 7.00)	5.00 (3.25 – 6.75)	1.50 (0.75 – 2.00)	0.945
Normalised In-degree	12.20 (4.87 – 17.07)	12.19 (7.93 – 16.46)	3.65 (1.83 – 4.87)	0.289
Out-degree	5.00 (4.00 – 7.50)	4.00 (3.00 – 6.50)	4.00 (2.25 – 9.25)	0.558
Normalised Out-degree	12.20 (9.76 – 18.29)	9.76 (7.32 – 15.85)	9.76 (5.49 – 22.00)	0.587
InClosesness	12.08 (7.78 – 12.88)	10.97 (10.32 – 12.23)	6.90 (1.50 – 7.88)	0.273
Normalised In-Closeness	29.50 (18.97 – 31.40)	27.27 (25.42 – 29.82)	16.84 (3.65 – 19.22)	0.273
OutCloseness	14.36 (12.41 – 15.10)	12.66 (5.00 – 14.46)	12.57 (3.00 – 17.25)	0.520
Normalised Out-Closeness	35.04 (30.27 – 36.78)	30.89 (12.20 – 35.35)	30.65 (7.32 – 42.07)	0.520
Between-ness	138.10 (87.20 – 181.70)	109.20 (36.10 – 189.20)	6.50 (0.00 – 53.20)	0.185
Normalised Between-ness	8.42 (5.32 – 11.07)	6.66 (2.20 – 11.53)	0.42 (0.00 – 3.25)	0.185

*Based on Kruskal-Wallis tests comparing variables among the three animal health wards. IQR – Interquartile range.

Table 5.6 Movement network patterns of cattle and goats within the three animal health wards of Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa during June 2018.

Variable	Cattle Median (IQR)	Goat Median (IQR)	P- value*
Degree centrality			
Out-degree	4.50 (3.00 – 7.00)	5.00 (3.00 – 7.00)	0.779
In-degree	2.00 (1.00 – 6.00)	3.00 (1.00 – 4.00)	0.435
Closeness centrality			
Out-closeness	14.10 (9.92 – 15.00)	14.58 (13.20 – 17.33)	0.021
In-closeness	8.50 (6.26 – 12.08)	7.92 (5.42 – 8.83)	0.056
Between-ness centrality	66.10 (10.00 – 146.30)	25.80 (0.00 – 79.10)	0.008

*Based on Wilcoxon signed-rank tests for comparing variables between the two species. IQR – Interquartile range.

Table 5.7 Foot-and-mouth disease vaccination coverage for cattle by communal dip tanks for the three animal health wards of Mnisi Tribal Authority, Bushbuckridge, Mpumalanga Province, South Africa for the period preceding the study (2017/2018).

Animal Health Ward/ dip tank	Number of cattle owners	Total number of cattle	Number of cattle vaccinated (%)	Vaccination interval days
Ward B1				
Clare B	95	1105	1010 (91.4)	242
Clare A	59	697	691 (99.1)	236
Welverdiend A	180	1793	1611 (89.8)	235
Welverdiend B	88	838	816 (97.3)	234
Tlhavekisa	75	538	478 (88.8)	233
Ward B2				
Eglington	126	950	687 (72.3)	222
Share	72	547	408 (74.6)	218
Utha (scheme)	10	316	314 (99.4)	217
Shorty	59	442	437 (98.8)	233
Athol	138	1024	629 (61.4)	232
Ward B3				
Gottenburg	117	1033	927 (89.7)	117
Utha A	88	830	788 (94.9)	116
Dixie	17	178	105 (58.9)	130
Seville B	57	779	764 (98.1)	108
Seville A	40	734	550 (74.9)	121
Hlalakahle	52	345	321 (93.0)	120

Supplemental Table 5.1 Relationship between the total population of goat owners within the study area and sample selection for interview during June 2018.

Dip tank	Number of goat owners	Goat population	Dip tank average flock size	Sample interviewed
Clare B	31	358	12	6
Clare A	29	219	8	5
Welverdiend A	8	94	12	6
Welverdiend B	26	314	12	6
Tlhavekisa	56	575	10	13
Eglington	65	398	6	15
Share	39	320	8	8
Shorty	32	310	10	6
Athol	31	370	12	6
Gottenburg	46	261	6	11
Utha A	48	273	6	9
Dixie	3	24	8	2
Seville B	54	327	6	11
Seville A	24	201	9	4
Hlalakahle	34	213	6	8

5.4 Discussion

The primary aim of this study was to evaluate the role that movement of livestock might play in the spread of FMD within a disease control area and identify high-risk disease locations for improved surveillance and strategic vaccination programmes. In this paper, the livestock movement networks were investigated because of our desire to improve FMD control in the country. It is expected that the results of this study will be useful for disease control through the implementation of risk-based surveillance and strategic vaccination within the FMD Protection zone of South Africa.

The number of goats kept by respondents in this study were similar to a smallholder study conducted in another part of the country (Braker et al., 2002). Most of the respondents from the three wards indicated subsistence and natural love for animals to be their major motivation for livestock farming. Improving disease control within the communal setting might improve the livelihoods of smallholder farmers because of this motivation. In addition to keeping goats, communal farmers also kept cattle and pigs, which are also susceptible hosts for FMD. Most communal farmers within the study area reported that they do not require livestock movement permits to move goats to neighboring and distant locations. This suggests a need to educate farmers concerning the risk of livestock movement out of disease control areas. Movement of live animals is one of the most common ways of spreading infectious diseases between holdings (Nremark et al., 2011). Most goats are bred and consumed locally within the communities with some reported movements outside the study area. In comparison, cattle have a more organized market through a local auctioneer and presumably most movements are issued permits by the local animal health authorities.

Most of the respondents indicated a natural love for animals (companionship) and subsistence as

the main motivators for keeping goats. This is similar to the findings of a previous study of communal farmers in South Africa where most respondents indicated companionship and subsistence as reasons for rearing goats (Braker et al., 2002). Respondents also indicated that goats fulfil important roles within their households as an easily convertible source of income in times of need. The rearing of livestock contributes to long-term savings and investment but only among a relatively small proportion of the respondents.

Most of the respondents kept goats and cattle in separate holdings during the night. This is contrary to the practice of pastoralists where livestock are collectively tethered together in common holdings (Lazarus et al., 2012). This might be due to differences in culture between respondents and pastoralists who are always on the move with their livestock and would find it difficult to separate livestock species. Farm biosecurity and proper husbandry management are fundamental to disease control (Correia-Gomes and Sparks, 2020; Megersa et al., 2009). Most of the farmers reported that they contact local veterinary services when animals are sick and that they would never sell sick animals. Respondents within the study area therefore have some animal health knowledge and access to local veterinary services. Feed scarcity is a major challenge for livestock production during drought within the study area. Respondents reported the use of communal resources for grazing and water, which is consistent with other studies conducted in communal settings (Nyaguthii et al., 2019; Rufael et al., 2008). Respondents within Wards I and II reported more use of supplemental feed possibly due to their proximity to a road network and urban communities with agricultural cooperative stores.

Livestock movement contributes to the spread of infectious diseases from endemic to free zones (Nremark et al., 2011). In South Africa, the majority of the country was previously classified as FMD free (DAFF, 2014). The KNP and adjoining nature reserves were classified as the FMD

Infected zone due to the existence of wildlife reservoirs including the African buffalo (*Syncerus caffer*) (DAFF, 2014). Communal farming areas surrounding the KNP were classified as the FMD Protection zone with vaccination, where cattle are routinely inspected and vaccinated against FMD (Lazarus et al., 2018). Movement of livestock from the FMD Protection zone to any other part of the country requires inspection and a movement permit (DAFF, 2014). In this study, respondents reported more movement of animals out of their holdings relative to movement into their holdings during the previous 12 months. Movement of cattle from this region is by official veterinary permit and sales are usually coordinated by a local auctioneer in collaboration with the local farming community. In this study, the most influential nodes for goat movements were communities closer to urban settlements with an accessible road network. The goat movement network had an overall density of 0.059, which reflects that the real movement patterns were only 5.9% compared to all possible movement within the network. Also, the centrality measures had low values suggesting that movement within the network had a random pattern. Eglington and Share, both in Ward II, had the highest out-degree centrality measures for goat movements, and thus were the most influential communities for the possible spread of diseases.

Gottenburg and Seville B, both in Ward III, were the most influential in the cattle movement network as demonstrated by their high out-degree centrality measures. The spread of infection is reportedly associated with out-degree centrality measures (Dubé et al., 2008). Consequently, the nodes with high out-degree centrality are spreaders of disease and are likely to increase the size of an epidemic. However, to prevent these nodes from spreading FMD, the local veterinary authority could give high priority for FMD control and vaccination within these communities. The cattle vaccination coverage for Eglington and Share for the period preceding the study was 72.3% and 74.6% respectively. However, the median (IQR) time since last vaccination for the first five nodes

with the highest out-degree centrality was 232 (220 – 233) days and this should be interpreted relative to the desired 120-day inter-vaccination interval. This prolonged interval has been previously reported from the study area (Lazarus et al., 2017). Cattle within these nodes have exceeded the expected vaccination interval and thus might have a high proportion of susceptible cattle.

Clare B had the highest in-degree centrality measure for the goat movement network followed by Eglington and Hlalakahle, which suggests high-risk nodes for disease outbreak occurrence. The cattle vaccination coverage for these nodes varied from 72.3 – 93%, but Eglington and Clare B had prolonged inter-vaccination intervals. Therefore, more FMD surveillance should be focused on these nodes during outbreaks as they tend to receive more in-ward animal movements relative to all other nodes. Eglington had a relatively high centrality measure for goat movement, and this might be due to its location and accessible road network compared to the more remote settings. Farmers in Eglington tended to source goats from nearby nodes and then export them to distant locations. A similar movement pattern was previously described in the study of traditional cattle trade network in Tak Province, Thailand (Khengwa et al., 2017). On average, the actors had a degree of 2.10 for both in-degree and out-degree which is quite low, given that there were 37 actors in the goat movement network in total. In the current network, the out-degree graph centralization of the goat movement network is much greater than the in-degree graph centralization, and this suggests that there is proportionally more out-degree movement in this network. These communities require education on disease prevention and control. In terms of the network analysis, such communities could be described as the disease spreaders. Therefore, in the event of an FMD outbreak within the study area, the relevant authorities should focus their disease control measures on nodes with higher out-degree centrality measures and middlemen involved in livestock

movements.

The two nodes with the highest in-degree centrality measures for the cattle movement network were nodes outside of the study area, Giyani in Limpopo Province and Thulamahashe in Mpumalanga Province. Both nodes are associated with urban development and organized abattoirs and butcheries. Middlemen are therefore key players in the movement of animals within this network. This is similar to a social network analysis of cattle movements in Kampong Cham, Kampong Speu and Takeo, Cambodia (Poolkhet et al., 2016). Implementing restrictions on trade activities and livestock movements from the nodes with high out-degree measures and the middlemen might limit the magnitude of disease spread to other nodes. In this network, farmers themselves could be effective disseminators of information to improve communication for the greater benefit of the network.

The between-ness centrality for the goat movement network was high for two nodes in Ward II and one node each in Wards I and III, reflecting the many steps connecting nodes to one another. Eglington and Shorty both in Ward II, Tlhavekisa in Ward I and Hlalakahle in Ward III, appear to be more important than the other nodes by this measure. This suggests that more animals pass through these nodes relative to the other nodes of the network. Interestingly, in 2017, a SAT2 FMD outbreak was reported in Tlhavekisa (Ward I), one of the communities with high between-ness centrality measure for the goat movement network (OIE-WAHID, 2017).

In 2019, two SAT2 FMD outbreaks (DAFF, 2019a; DAFF, 2019b) were reported in the former FMD Free Zone of Limpopo Province, which were linked to animal movements but not associated with the study area. However, on the 3rd of March 2020, a SAT2 FMD outbreak was reported at Gottenburg, Seville B and Utha A (Ward III) which subsequently spread to Tlhavekisa and Clare B (Ward I) by the second week of April (Mr Solly Mokone, Animal Health Technician personal

communication). From our study, Gottenburg and Seville B were the most influential nodes in cattle movements as demonstrated by their out-degree centrality measures. The rapid spread from the three initial villages is therefore consistent with the expectations based on the current findings.

Goats have been described to be “silent shedders” of FMD without showing obvious clinical and sickness behaviors. This therefore makes it very difficult to identify infected goats that might pose threat for the spread of disease through animal movement. The results of this study indicate that goats are moved by communal farmers out of the study area without official movement permits, although, the absolute number of movements appears to be low. The study also identified communities at high risk of disease occurrence and communities that might play important roles in subsequent disease spread. Four locations in the (former) FMD Free zone of the country (Nelspruit, Tzaneen, Barbertone and Leboeng) were identified as having connections with movement of goats from the study area and this calls for careful monitoring to mitigate the potential spread of FMD from the study area.

5.5 Limitations of this study

The results of this study should be interpreted considering several limitations. A goat identification system and an organized database are not in use within the study area. Therefore, it was not possible to verify the movement of individual animals. Animal movement data derived in this study was solely based on interviews and subject to recall bias and purposeful misinformation. We were unable to follow up each origin and destination reported by the respondents outside of the study area due to limited budgets and resources. Other limitations to the study include the small study area, small sample size and the lack of production records for the verification of animal movements.

5.6 Conclusions and recommendations

In this study, the results suggest the need to improve control measures in the study area. We recommend the following: control goat movement via the official movement permit system, establish an organized goat auction point or market to control trader activities, initiate a traceable livestock identification system, develop a database for livestock movements and improve surveillance and inspection of FMD in goats within the FMD Protection zone.

Cattle entering communities with the high in-degree measures should be properly inspected and ensured they originated from herds that have been vaccinated against FMD with evidence of branding or stock card record and official movement permit. Farmers moving cattle from the communities with the high out-degree measures should ensure that their animals are vaccinated against FMD and that they are inspected and issued official movement permits in compliance with the local disease control policy. Presented information could be used to improve FMD control within the study area and the progressive control of FMD in general by adoption in other rural settings of southern Africa.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

The studies presented in this thesis were implemented in an effort to understand the role of goats in the epidemiology of foot-and-mouth disease (FMD) within the protection zone of South Africa. Prior to this study, no information was available on the role of goats in the epidemiology of FMD or their response to FMD vaccination within South Africa.

Clinical surveillance, vaccination and movement control are fundamental tools for the effective control of FMD and other transboundary animal diseases in endemic countries. Cattle are the livestock species most commonly included in prophylactic vaccination programmes for the control of FMD. However, in some situations, sheep and goats are vaccinated during active outbreaks. In South Africa, goats are not currently included in the routine FMD mass vaccination campaigns.

FMD is a controlled animal disease in the Republic of South Africa as defined in Section 1 of the Animal Diseases Act, 1984 (Act No. 35 of 1984) and prescribed control measures are included in Section 9 of the Act (Animal Diseases Regulation, No. R 2026 of 26 September 1986, Table 2, as amended). The Veterinary Procedural Notice (VPN) for Foot-and-Mouth Disease Control in South Africa was developed as a guiding document to address control measures in more detail (DAFF, 2014).

The VPN-FMD defines measures for FMD control in South Africa in an effort to protect the FMD-free status and restrict infection within the FMD infected zones of the country (DAFF, 2014). This addresses the movement of cloven-hoofed animals and their products according to the following principles; a) prohibition of high-risk movements, b) facilitation of medium-risk movement by

implementation of appropriate risk mitigation measures and c) promotion of negligible risk movements by the application of commodity-based trade principles. The VPN as mandated by the Animal Diseases Act, 1984 (Act, No. 35 of 1984), also makes provision for three key control measures: clinical surveillance, vaccination and movement control.

6.1.1 Clinical surveillance

The VPN states that all cattle within the protection zones with vaccination must be inspected every 7 days and all small stock (i.e. goats, sheep and pigs) must be inspected every 28 days (DAFF, 2014). Movement of livestock within the protection zone is only allowed from farms and communal dip tanks where turnout and frequency (the whole dip tank and the herd) have been at least 50% within the previous month. Routine mouth examinations should 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. Sheep and goats within the protection zone with vaccination are currently inspected every 28 days with details of flock increase or decrease and movements entered into both the veterinary services' stock register and the farmer's stock card. Small stock are not subjected to mouth or feet examination during inspection and in most cases, animal owners do not comply with the registration of their small stock. It would be a difficult task to conduct mouth examinations in all goats due to their overwhelming population in some communities. However, since inspection is performed on a monthly basis, randomly selecting at least 10 goats out of a village, as prescribed for cattle, would improve clinical surveillance of FMD in the communal areas. It is often assumed that goats are not important in the epidemiology of FMD in endemic settings, as the clinical signs of FMD in this species are typically mild or inapparent (Arzt et al., 2011a; Kitching and Hughes, 2002). However, there is evidence that these species play a role in

the epidemiology of FMD in Africa (Balinda et al., 2009; Lazarus et al., 2012; Paton et al., 2009). The research performed in Chapter 3 of this thesis has shown that indigenous South African goats can be infected with SAT1 FMDV, and not always present with obvious clinical signs. This presents a challenge for the clinical surveillance of FMD within the protection zones, since goats do not look sick and farmers will not be able to recognize an infected flock. The results presented in Chapter 3 also demonstrated natural contact transmission between animals, meaning that sub-clinically infected goats might be able to transmit infection under field situations. This therefore calls for the improved surveillance of FMD in this species, since officials inspecting affected flocks will not see lameness or excessive salivation as is frequently observed in cattle. The results presented in Chapter 3 suggest that the most common lesion observed in goats are erosions and ulcers of the buccal surface of the lips that are mild and do not look like the "typical" FMD lesions of cattle. Therefore, when not properly trained to identify such lesions, authorities inspecting goats for the signs of FMD might easily miss or disregard these features. In Chapter 4, only 60% of our unvaccinated but experimentally infected goats presented with secondary lesions of FMD one-week post-challenge. Therefore, when inspecting goats for clinical signs of FMD in the field the proportion of animals presenting with these signs might be low in the flock and careful inspection will be required to identify infected animals. Antibodies to the FMD 3ABC non-structural proteins were detected in our study animals as early as 7 days post-challenge and this assay along with improved clinical surveillance in the field might enhance our capacity for FMD control within disease endemic settings.

During a recent SAT2 FMD outbreak within the formerly FMD free zone of Limpopo Province, we were able to identify FMDV seropositive goats in some households using solid-phase competition ELISA (SPCE). However, flocks containing goats with FMD-like lesions were

completely seronegative by SPCE. Although, these suspect FMD oral lesions were similar to the observations from our challenge study using SAT1 FMDV. Also, viral RNA was detected from a subset of goats with suspect lesions and some goats in seropositive flocks had oral lesions suggestive of healed FMD erosions/ulcers (data unpublished).

6.1.2 Vaccination

Vaccination is widely applied to prevent, control and eradicate FMD (Bergmann et al., 2005; Garland, 1999). However, cattle are most commonly vaccinated with other susceptible domestic cloven-hoofed livestock species not usually included in prophylactic vaccination campaigns. The South African VPN prescribes that all cattle within the protection zone with vaccination, irrespective of age, be vaccinated every 4 months including a re-vaccination after 3-4 weeks for cattle having just received their first vaccination (DAFF, 2014). Vaccination dates, herd identities and number of cattle vaccinated must be recorded accurately in veterinary service cattle registers at each round of vaccination. Currently, goats are not included in the FMD mass vaccination campaigns. Goats should be considered to be included in mass vaccination campaigns within high-risk locations, especially when outbreaks have been reported. However, we must consider the expected annual turnover rate in goat production and the need to ensure each animal is identified by tattoo as recommended by the Animal Identification Act (Act No. 6 of 2002). The feasibility concerning cost and logistics associated with the implementation of such a programme is currently unknown. Preliminary findings from Chapter 4 suggest that goats vaccinated with one-third ($1/3^{rd}$) of the full dose high potency ($>6PD_{50}$) vaccine were clinically protected from disease and had evidence of reduced viral shedding. Vaccinating goat populations at risk during an outbreak in cattle might help control the spread of FMD within the protection zone. Cattle within the protection zone are handled and vaccinated in crush pens at the communal dip tanks, which facilitates

logistics. However, considering the goat husbandry system within the protection zones and the population of goats that will be included in this programme, vaccination should be considered at a household level. Additional workforce will be required to compliment the already strained provincial resources and there will also be a need for improved biosecurity to prevent FMDV transmission by vaccinators. Smallholder goat farmers would also need education on the need to include goats in the on-going mass vaccination campaign programmes, to improve disease control. With the evidence of natural transmission occurring between in-contact goats in our study, there is the need to ensure all susceptible animals within the protection zone have sufficient herd immunity during high risk seasons. Vaccination of goats could be an option to improve FMD control in endemic settings. The decision to use vaccination is a national responsibility that should be carefully considered.

6.1.3 Movement control

Written FMD inter State Veterinary/ inter zonal approval must be obtained from the relevant authorities for all movement of live cloven-hoofed animals (except buffalo, which have separate regulations) between different State Veterinary areas or between different FMD zones (DAFF, 2014). All animals and products permitted to move must have a similar FMD risk status as the destination zone/area. The Animal Identification Act (Act No 6, of 2002) recommends that all small stock including goats are tattooed at the age of one month for proper identification and traceability. The register of identification kept in terms of Section 6 of the Act must contain particulars regarding: a) the identification mark allocated in terms of Section 5 of the Act, b) the full name, identity number, permanent and physical address of the owner of such mark and c) the date on which the identification mark was issued. Personal experience suggests that goats within our study area are not commonly identified as recommended by the Animal Identification Act.

Findings from Chapter 5 indicate that cattle and goats are moved out of the study area to the disease-free zone of the country either for consumption or husbandry purposes. However, farmers in the study only apply for official veterinary movement permits to move cattle. Most farmers reported that official movement permits are not required for goats. Results demonstrated that cattle are moved officially to government approved abattoirs for slaughter within the disease-free zones after purchase at a government organized auction within the protection zone. Farmers also indicated that one contributing factor for the movement of animals out of the protection zone is the lack of market access due to disease control policy. The current results indicated that most movements of goats are within the protection zone for local consumption and traditional rituals. However, some people move goats out of the protection zone to disease free zones without official veterinary movement permits. This suggests that goats might be silent spreaders of FMD from the protection zone should an outbreak occur. Although, the VPN prescribes the issuance of official veterinary movement permits for all animals at risk and their products from the protection zone, most farmers were unaware about this disease control measure. Therefore, there is a need to engage farmers within the protection zones concerning the risk associated with unpermitted movement of animals into the FMD free zone of the country. To improve our strategy for the control of FMD in endemic settings, there should be adequate enforcement of official movement control and this should include goats and other susceptible livestock at risk.

6.2 Conclusions

The control of FMD in most parts of Africa remains a challenge. There is a need for improved clinical surveillance in all susceptible livestock species at risk, cost-effective vaccination programmes and enlightened movement control policies. Understanding FMDV infection in goats provides an opportunity to facilitate FMD endemic countries within the SADC region and Africa

to progressively reduce disease impact and FMDV transmission. Data from this study might serve as a model for the control of FMD using the progressive control programme for foot-and-mouth disease (PCP-FMD) pathway as recommended by the OIE. This study has demonstrated that goats can be infected with FMDV SAT1 and transmit infection to in-contact goats. Therefore, to improve the progressive control of FMD in disease endemic countries, goats should be included in clinical surveillance programmes and inspections. Vaccination of goats and evaluation of the movement network of all susceptible livestock within the SADC and Africa will also provide further information for countries to advance on the PCP-FMD pathway. Countries should put in place FMD monitoring and evaluation systems to measure the effectiveness of control programmes. Information from these studies should be used to inform policy with the goal of improved FMD surveillance, livestock vaccination and inspection for a regional FMD control initiative within the SADC and Africa as a whole.

6.3 Recommendations

The results of this thesis can be used to generate several recommendations for FMD control in South Africa, specifically related to clinical surveillance, vaccination and movement control policies. Findings demonstrated that goats can be infected with FMDV and then transmit infection through direct contact without displaying obvious clinical signs. We recommend that when inspecting goats for suspected FMDV infections, attention should be focused on the oral mucosa of the lips and gums in addition to the tongue. Official veterinarians and animal health technicians providing services within endemic settings should be trained on the clinical investigation of FMD lesions in goats to enhance their capacity for improved surveillance and inspection. We also advocate that regular serological surveillance for FMD be conducted in goats within endemic settings. There is also a need for continued investigation of the role goats play in the epidemiology and maintenance of FMDV under field conditions within the Southern African Development Community (SADC).

To ensure sufficient herd immunity in all susceptible livestock within endemic settings, we recommend the inclusion of small ruminants (goats and sheep) in prophylactic mass vaccination campaigns. As a quality assurance tool for the effective control of FMD, we also recommend that post-vaccination monitoring programmes be implemented after every campaign. It will also be beneficial to routinely conduct vaccine-matching on outbreak samples. This will determine the effectiveness of the vaccination campaign for presentation to the relevant authorities and vaccine manufacturers. Farmers or communities with good vaccination coverage during any vaccination round should be acknowledged and rewarded by provision of essential amenities. These could include water boreholes (wells) and animal health incentives at dip tanks to recognize their efforts in national disease control.

Animal movement is a cause of infectious disease spread between holdings and communities. This study has been able to identify several high-risk hotspots within the study area based on network movement data reported by smallholder farmers. We therefore recommend the following measures: include goat movement in official veterinary movement permit systems, establish organized goat auction points or markets to control trader activities in high-risk locations, develop traceable livestock identification systems and create databases of livestock movements.

6.4 Further research priorities

Further research should be directed towards understanding the epidemiology of SAT FMDV in goats under field situations within the SADC. Other breeds of goats should be included in future research since exotic breeds are considered more susceptible to FMD compared to animals that are indigenous to endemic areas. The expansion of these current studies have the aim of addressing the current problems of FMD within the SADC as a whole.

The current SPCE assay should be validated for use in goats because the cutoff used for the detection of bovine antibodies might not be suitable for small ruminants. Different FMD vaccination schedules need to be evaluated to identify a realistic and optimal vaccination regimen that can be applicable in the field. The vaccine manufacturers recommend that goats be vaccinated using ½ the cattle dose for oil-emulsion vaccines and be re-vaccinated at 3-4 weeks with subsequent boosters every 4-6 months. However, this is difficult to achieve in the field and modifications of this vaccination schedule should be evaluated. The effect of the vaccine preparation on virus replication and duration of immunity after vaccination in goats also needs to be considered as a future research priority. The current studies only evaluated the effect of vaccination on clinical disease and viral shedding. There is also the need to study the persistence of FMDV in goats with the aim of understanding their possible role in disease transmission in

endemic settings.

To identify FMD high-risk locations within the country, a network analysis study of all susceptible livestock within the entire protection zone should be performed. The identification of high-risk nodes could be further substantiated using sero-surveillance data, post-vaccination sero-monitoring or the collection of clinical specimens for detection of virus. This will provide information to develop strategic surveillance and targeted vaccination programmes.

REFERENCE

Abd El Wahed, A., El-Deeb, A., El-Tholoth, M., Abd El Kader, H., Ahmed, A., Hassan, S., Hoffmann, B., Haas, B., Shalaby, M.A., Hufert, F.T., Weidmann, M., 2013. A Portable Reverse Transcription Recombinase Polymerase Amplification Assay for Rapid Detection of Foot-and-Mouth Disease Virus. PLoS ONE 8. https://doi.org/10.1371/journal.pone.0071642

Abdul-Hamid, N.F., Hussein, N.M., Wadsworth, J., Radford, A.D., Knowles, N.J., King, D.P., 2011. Phylogeography of foot-and-mouth disease virus types O and A in Malaysia and surrounding countries. Infection, Genetics and Evolution. https://doi.org/ 10.1016/j.meegid.2010.11.003

Alexandersen, S, Brotherhood, I., Donaldson, A.I., 2002. Natural aerosol transmission of foot-and-mouth disease virus to pigs: minimal infectious dose for strain O1 Lausanne. Epidemiology and infection 128, 301–312.

Alexandersen, S., Donaldson, A.I., 2002. Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. Epidemiology and Infection 128, 313–323. https://doi.org/10.1017/S0950268801006501

Alexandersen, S., Kitching, R.P., Mansley, L.M., Donaldson, A.I., 2003a. Clinical and laboratory investigations of five outbreaks of foot-and-mouth disease during the 2001 epidemic in the United Kingdom. Veterinary Record. https://doi.org/10.1136/vr.152.16.489

Alexandersen, Soren, Zhang, Z., Donaldson, A.I., 2002a. Aspects of the persistence of foot-and-mouth disease virus in animals--the carrier problem. Microbes and infection Institut Pasteur 4, 1099–1110.

Alexandersen, Soren, Zhang, Z., Donaldson, A.I., 2002b. Aspects of the persistence of foot-and-

mouth disease virus in animals - The carrier problem. Microbes and Infection 1099–1110. https://doi.org/10.1016/S1286-4579(02)01634-9

Alexandersen, S., Zhang, Z., Donaldson, A.I., Garland, A.J.M., 2003b. The pathogenesis and diagnosis of foot-and-mouth disease. Journal of Comparative Pathology 129, 1–36. https://doi.org/10.1016/S0021-9975(03)00041-0

Anderson, E.C., Doughty, W.J., Anderson, J., 1976. The role of sheep and goats in the epizootiology of foot-and-mouth disease in Kenya. The Journal of hygiene 76, 395–402. https://doi.org/10.1017/S0022172400055315

Anonymous, 2019. Goat Farming in South Africa [WWW Document]. Online publication. URL http://southafrica.co.za/goats-in-south-africa.html (accessed 3.2.20).

Anonymous, 2014. 27.6.2014. Official Journal of the European Union 2014, 33–49.

Araújo, J.P., Montassier, H.J., Pinto, A.A., 1996. Liquid-phase blocking sandwich enzyme-linked immunosorbent assay for detection of antibodies against foot-and-mouth disease virus in water buffalo sera. American Journal Of Veterinary Research 57, 840–843.

Arzt, J., Baxt, B., Grubman, M.J., Jackson, T., Juleff, N., Rhyan, J., Rieder, E., Waters, R., Rodriguez, L.L., 2011a. The Pathogenesis of Foot-and-Mouth Disease II: Viral Pathways in Swine, Small Ruminants, and Wildlife; Myotropism, Chronic Syndromes, and Molecular Virus-Host Interactions. Transboundary and Emerging Diseases 58, 305–326.

Arzt, J., Juleff, N., Zhang, Z., Rodriguez, L.L., 2011b. The Pathogenesis of Foot-and-Mouth Disease I: Viral Pathways in Cattle. Transboundary and Emerging Diseases 58, 291–304. https://doi.org/10.1111/j.1865-1682.2011.01204.x

Aznar, M.N., Stevenson, M.A., Zarich, L., León, E.A., 2011. Analysis of cattle movements in Argentina, 2005. Preventive Veterinary Medicine 98, 119–127. https://doi.org/10.1016/j.prevetmed.2010.11.004

Bachanek-Bankowska, K., Mero, H.R., Wadsworth, J., Mioulet, V., Sallu, R., Belsham, G.J., Kasanga, C.J., Knowles, N.J., King, D.P., 2016. Development and evaluation of tailored specific real-time RT-PCR assays for detection of foot-and-mouth disease virus serotypes circulating in East Africa. Journal of Virological Methods. https://doi.org/10.1016/j.jviromet.2016.08.002

Bajardi, P., Barrat, A., Savini, L., Colizza, V., 2012. Optimizing surveillance for livestock disease spreading through animal movements. Journal of the Royal Society Interface 9, 2814–2825. https://doi.org/10.1098/rsif.2012.0289

Balinda, S.N., Ayebazibwe, C., Mwiine, F.N., Tjørnehøj, K., Alexandersen, S., 2012. Application of the ceditest® FMDV type O and FMDV-NS enzyme-linked immunosorbent assays for detection of antibodies against Foot-and-mouth disease virus in selected livestock and wildlife species in Uganda. Journal of Veterinary Diagnostic Investigation 24, 270–276. https://doi.org/10.1177/1040638711435807

Balinda, S.N., Tjørnehøj, K., Muwanika, V.B., Sangula, A.K., Mwiine, F.N., Ayebazibwe, C., Masembe, C., Siegismund, H.R., Alexandersen, S., 2009. Prevalence estimates of antibodies towards foot-and-mouth disease virus in small ruminants in Uganda. Transboundary and Emerging Diseases 56, 362–371.

Baranowski, E., Ruiz-Jarabo, C.M., Sevilla, N., Andreu, D., Beck, E., Domingo, E., 2000. Cell Recognition by Foot-and-Mouth Disease Virus That Lacks the RGD Integrin-Binding Motif:

Flexibility in Aphthovirus Receptor Usage. Journal of Virology. https://doi.org/10.1128/jvi.74.4.1641-1647.2000

Barnett, P. V., Carabin, H., 2002. A review of emergency foot-and-mouth disease (FMD) vaccines. Vaccine. https://doi.org/10.1016/S0264-410X(01)00503-5

Barnett, P. V., Cox, S.J., Aggarwal, N., Gerber, H., McCullough, K.C., 2002. Further studies on the early protective responses of pigs following immunisation with high potency foot and mouth disease vaccine. Vaccine 20, 3197–3208. https://doi.org/10.1016/S0264-410X(02)00242-6

Barnett, P.V. V., Cox, S.J.J., 1999. The Role of Small Ruminants in the Epidemiology and Transmission of Foot-and-Mouth Disease. Veterinary Journal London England 158, 6–13. https://doi.org/10.1053/tvjl.1998.0338

Barteling, S.J., Vreeswijk, J., 1991. Developments in foot-and-mouth disease vaccines. Vaccine 9, 75–88. https://doi.org/10.1016/0264-410X(91)90261-4

Bastos, A.D.S., Boshoff, C.I., Keet, D.F., Bengis, R.G., Thomson, G.R., 2000. Natural transmission of foot-and-mouth disease virus between African buffalo (Syncerus caffer) ans impala (Aepyceros melampus) in the Kruger National Park, South Africa. Epidemiology and Infection 124, 591–598. https://doi.org/10.1017/S0950268899004008

Bastos, A.D.S., Haydon, D.T., Forsberg, R., Knowles, N.J., Anderson, E.C., Bengis, R.G., Nel, L.H., Thomson, G.R., 2001. Genetic heterogeneity of SAT-1 type foot-and-mouth disease viruses in southern Africa. Archives of Virology 146, 1537–1551.

Bastos, A.D.S., Haydon, D.T., Sangaré, O., Boshoff, C.I., Edrich, J.L., Thomson, G.R., 2003. The

implications of virus diversity within the SAT 2 serotype for control of foot-and-mouth disease in sub-Saharan Africa. Journal of General Virology 84, 1595–1606.

Bates, T.W., Thurmond, M.C., Carpenter, T.E., 2001. Direct and indirect contact rates among beef, dairy, goat, sheep, and swine herds in three California counties, with reference to control of potential foot-and-mouth disease transmission. American Journal of Veterinary Research 62, 1121–1129.

Bautista, E.M., Ferman, G.S., Golde, W.T., 2003. Induction of lymphopenia and inhibition of T cell function during acute infection of swine with foot and mouth disease virus (FMDV). Veterinary Immunology and Immunopathology. https://doi.org/10.1016/S0165-2427(03)00004-7

Belsham, G.J., 2005. Translation and replication of FMDV RNA. Current Topics in Microbiology and Immunology 288, 43–70.

Bengis, R.G., Kock, R.A., Fischer, J., 2002. Infectious animal diseases: the wildlife/livestock interface. Revue scientifique et technique (International Office of Epizootics) 21, 53–65.

Bergmann, I.E., Malirat, V., Falczuk, A.J., 2005. Evolving perception on the benefits of vaccination as a foot and mouth disease control policy: Contributions of South America. Expert Review of Vaccines. https://doi.org/10.1586/14760584.4.6.903

Bhat, S.A., Saravanan, P., Hosamani, M., Basagoudanavar, S.H., Sreenivasa, B.P., Tamilselvan, R.P., Venkataramanan, R., 2013. Novel immunogenic baculovirus expressed virus-like particles of foot-and-mouth disease (FMD) virus protect guinea pigs against challenge. Research in Veterinary Science. https://doi.org/10.1016/j.rvsc.2013.07.007

Bhebhe, B., Vhoko, K., Tshabalala, P., Makaya, P V., Mdlongwa, E., Pfukenyi, D.M., 2016. Seroprevalence of foot-and-mouth disease in goats from Matebeleland South Province of Zimbabwe. Zimbabwe Veterinary Journal 34, 1–4.

Bigras-Poulin, M., Barfod, K., Mortensen, S., Greiner, M., 2007. Relationship of trade patterns of the Danish swine industry animal movements network to potential disease spread. Preventive Veterinary Medicine 80, 143–165.

Bigras-Poulin, M., Thompson, R.A., Chriel, M., Mortensen, S., Greiner, M., 2006. Network analysis of Danish cattle industry trade patterns as an evaluation of risk potential for disease spread. Preventive Veterinary Medicine 76, 11–39.

Biswal, J.K., Jena, S., Mohapatra, J.K., Bisht, P., Pattnaik, B., 2014. Detection of antibodies specific for foot-and-mouth disease virus infection using indirect ELISA based on recombinant nonstructural protein 2B. Archives of Virology 159, 1641–1650. https://doi.org/10.1007/s00705-013-1973-3

Blignaut, B., van Heerden, J., Reininghaus, B., Fosgate, G.T., Heath, L., 2020. Characterization of SAT2 foot-and-mouth disease 2013/2014 outbreak viruses at the wildlife–livestock interface in South Africa. Transboundary and Emerging Diseases. https://doi.org/10.1111/tbed.13493

Boehm, U., Klamp, T., Groot, M., Howard, J.C., 1997. Cellular response to Interferon-γ. Annual Review of Immunology 15, 749–795. https://doi.org/10.1146/annurev.immunol.15.1.749

Boender, G.J., Hagenaars, T.J., Bouma, A., Nodelijk, G., Elbers, A.R.W., De Jong, M.C.M., Van Boven, M., 2007. Risk maps for the spread of highly pathogenic avian influenza in poultry. PLoS Computational Biology 3, 704–712.

Borgatti SP, Everett MG, J.J., 2013. Analyzing social networks. Thousand Oaks: Sage.

Braker, M.J.E., Udo, H.M.J., Webb, E.C., 2002. Impacts of intervention objectives in goat production within subsistence farming systems in South Africa. South African Journal of Animal Sciences 32, 185–191. https://doi.org/10.4314/sajas.v32i3.3745

Bravo de Rueda, C., Dekker, A., Eblé, P.L., de Jong, M.C.M., 2014. Identification of factors associated with increased excretion of foot-and-mouth disease virus. Preventive Veterinary Medicine 113, 23–33. https://doi.org/10.1016/j.prevetmed.2013.10.005

Brehm, K.E., Ferris, N.P., Lenk, M., Riebe, R., Haas, B., 2009. Highly Sensitive Fetal Goat Tongue Cell Line for Detection and Isolation of Foot-and-Mouth Disease Virus. Journal of Clinical Microbiology 47, 3156–3160. https://doi.org/10.1128/JCM.00510-09

Brito, B.P., Jori, F., Dwarka, R., Maree, F.F., Heath, L., Perez, A.M., 2016. Transmission of foot-and-mouth disease SAT2 viruses at the wildlife-livestock interface of two major transfrontier conservation areas in Southern Africa. Frontiers in Microbiology 7, 528. https://doi.org/10.3389/fmicb.2016.00528

Brito, B.P., Rodriguez, L.L., Hammond, J.M., Pinto, J., Perez, A.M., 2017. Review of the Global Distribution of Foot-and-Mouth Disease Virus from 2007 to 2014. Transboundary and Emerging Diseases 64, 316–332. https://doi.org/10.1111/tbed.12373

Brocchi, E., Bergmann, I.E., Dekker, A., Paton, D.J., Sammin, D.J., Greiner, M., Grazioli, S., De Simone, F., Yadin, H., Haas, B., Bulut, N., Malirat, V., Neitzert, E., Goris, N., Parida, S., Sørensen, K., De Clercq, K., 2006. Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. Vaccine 24, 6966–6979. https://doi.org/10.1016/j.vaccine.2006.04.050

Burrows, R., 1968. The persistence of foot-and mouth disease virus in sheep. The Journal of Hygiene 66, 633–40. https://doi.org/10.1017/S0022172400028369

Callahan, J.D., Brown, F., Osorio, F.A., Sur, J.H., Kramer, E., Long, G.W., Lubroth, J., Ellis, S.J., Shoulars, K.S., Gaffney, K.L., Rock, D.L., Nelson, W.M., 2002. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. Journal of American Veterinary Medical Assocsociation 220, 1636–1642.

Callens, M., De Clercq, K., 1997. Differentiation of the seven serotypes of foot-and-mouth disease virus by reverse transcriptase polymerase chain reaction. Journal of Virological Methods. https://doi.org/10.1016/S0166-0934(97)00074-8

Capozzo, A. V., Wilda, M., Bucafusco, D., de los Ángeles Lavoria, M., Franco-Mahecha, O.L., Mansilla, F.C., Pérez-Filgueira, D.M., Grigera, P.R., 2011. Vesicular Stomatitis Virus glycoprotein G carrying a tandem dimer of Foot and Mouth Disease Virus antigenic site A can be used as DNA and peptide vaccine for cattle. Antiviral Research. https://doi.org/10.1016/j.antiviral.2011.08.006

Cardassis, J., Pappous, C., Brovas, D., Strouratis, P., Seimenis, A., 1966. Test of infectivity and dosage of foot and mouth disease vaccine in sheep. Bulletin de Office International des Epizooties 65, 427–438.

Cedillo-Barrón, L., Foster-Cuevas, M., Belsham, G.J., Lefèvre, F., Parkhouse, R.M.E., 2001. Induction of a protective response in swine vaccinated with DNA encoding foot-and-mouth disease virus empty capsid proteins and the 3D RNA polymerase. Journal of General Virology. https://doi.org/10.1099/0022-1317-82-7-1713

Chan, Y.H., 2003. Randomised controlled trials (RCTS) - Sample size: The magic number?

Singapore Medical Journal 44, 172–174.

Chapman, W.G., Ramshaw, I.A., 1971. Growth of the IB-RS-2 Pig Kidney Cell Line in Suspension Culture and Its Susceptibility to Foot-and-Mouth Disease Virus. Applied Microbiology 22, 1–5.

Chen, H. T., Zhang, J., Liu, S., Liu, X.T., Chen, H., Zhang, J., Liu, Y., Liu, X., 2011. Detection of foot-and-mouth disease virus RNA by reverse transcription loop-mediated isothermal amplification. Virology Journal 8, 510. https://doi.org/10.1186/1743-422X-8-510

Chen, H.T., Peng, Y.H., Zhang, Y.G., Liu, X.T., 2013. Detection of foot-and-mouth disease serotype O by ELISA using a monoclonal antibody. Monoclonal Antibodies in Immunodiagnosis and Immunotherapy 32, 47–49. https://doi.org/10.1089/mab.2012.0081

Chinazzi, M., Davis, J.T., Ajelli, M., Gioannini, C., Litvinova, M., Merler, S., y Piontti, A.P., Mu, K., Rossi, L., Sun, K., 2020. The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID-19) outbreak. Science 369, 394–400.

Chinsangaram, J., Beard, C., Mason, P.W., Zellner, M.K., Ward, G., Grubman, M.J., 1998. Antibody Response in Mice Inoculated with DNA Expressing Foot-and-Mouth Disease Virus Capsid Proteins. Journal of Virology 72, 4454–4457. https://doi.org/10.1128/jvi.72.5.4454-4457.1998

Chitray, M., Grazioli, S., Willems, T., Tshabalala, T., De Vleeschauwer, A., Esterhuysen, J.J., Brocchi, E., De Clercq, K., Maree, F.F., 2018. Development and validation of a foot-and-mouth disease virus SAT serotype-specific 3ABC assay to differentiate infected from vaccinated animals. Journal of Virological Methods 255, 44–51. https://doi.org/10.1016/j.jviromet.2018.02.006

Christensen, J., McNab, B., Stryhn, H., Dohoo, I., Hurnik, D., Kellar, J., 2008. Description of empirical movement data from Canadian swine herds with an application to a disease spread simulation model. Preventive Veterinary Medicine 83, 170–185.

Christley, R.M., Pinchbeck, G.L., Bowers, R.G., Clancy, D., French, N.P., Bennett, R., Turner, J., 2005. Infection in social networks: Using network analysis to identify high-risk individuals. American Journal of Epidemiology 162, 1024–1031.

Cloete, M., Dungu, B., Van Staden, L.I., Ismail-Cassim, N., Vosloo, W., 2008. Evaluation of different adjuvants for foot-and-mouth disease vaccine containing all the SAT serotypes. The Onderstepoort Journal of Veterinary Research 75, 17–31. https://doi.org/10.4102/ojvr.v75i1.84

Collen, T., 1994. Foot-and-mouth disease (Aphthovirus): viral T cell epitopes, in: Goddeevis, I.M.B.M.L. (Ed.), Cell-Mediated Immunity in Ruminants. Boca Raton: CRC Press, pp. 173–212.

Correia-Gomes, C., Sparks, N., 2020. Exploring the attitudes of backyard poultry keepers to health and biosecurity. Preventive Veterinary Medicine 174, 104812. https://doi.org/10.1016/j.prevetmed.2019.104812

Cox, S.J., Barnett, P. V., Dani, P., Salt, J.S., 1999. Emergency vaccination of sheep against foot-and-mouth disease: Protection against disease and reduction in contact transmission. Vaccine 17, 1858–1868. https://doi.org/10.1016/S0264-410X(98)00486-1

Cubillos-Zapata, C., Guzman, E., Turner, A., Gilbert, S.C., Prentice, H., Hope, J.C., Charleston, B., 2011. Differential Effects of Viral Vectors on Migratory Afferent Lymph Dendritic Cells In Vitro Predict Enhanced Immunogenicity In Vivo. Journal of Virology 85, 9385–9394.

https://doi.org/10.1128/jvi.05127-11

DAFF, 2019. National Livestock Statistics. Pretoria.

DAFF 2014, 2014. Veterinary Procedural Notice for FMD Control in South Africa. Pretoria.

DAFF 2019a, 2019. Positive foot-and-mouth disease results in the Vhembe District of Limpopo. Pretoria.

DAFF 2019b, 2019. Press Release: Positive foot-and-mouth disease result in the Molemole District, Limpopo, South Africa. Pretoria.

De la Rua R., Watkin G. H., W.P.J., 2001. Idiopathic mouth ulcers in sheep. Veterinary Record 149, 30–31.

Denkers, E.Y., Gazzinelli, R.T., 1998. Regulation and function of T-cell-mediated immunity during Toxoplasma gondii infection. Clinical Microbiology Reviews 11, 569–588. https://doi.org/10.1128/cmr.11.4.569

Di Nardo, A., Knowles, N.J., Paton, D.J., 2011. Combining livestock trade patterns with phylogenetics to help understand the spread of foot and mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia. OIE Revue Scientifique et Technique 30, 63–85. https://doi.org/10.20506/rst.30.1.2022

Di Nardo, A., Knowles, N.J., Wadsworth, J., Haydon, D.T., King, D.P., 2014. Phylodynamic reconstruction of O CATHAY topotype foot-and-mouth disease virus epidemics in the Philippines. Veterinary Research 45. https://doi.org/10.1186/s13567-014-0090-y

Diaz-San Segundo, F., Medina, G.N., Stenfeldt, C., Arzt, J., de los Santos, T., 2017. Foot-and-mouth disease vaccines. Veterinary Microbiology 206, 102–112. https://doi.org/

10.1016/j.vetmic.2016.12.018

Diaz-San Segundo, F., Salguero, F.J., de Avila, A., Fernandez de Marco, M.M., Sanchez-Martin, M.A., Sevilla, N., 2006. Selective Lymphocyte Depletion during the Early Stage of the Immune Response to Foot-and-Mouth Disease Virus Infection in Swine. Journal of Virology 80, 2369–2379. https://doi.org/10.1128/jvi.80.5.2369-2379.2006

Diego, M. De, Brocchi, E., Mackay, D., Simone, F. De, 1997. The non-structural polyprotein 3ABC of foot-and-mouth disease virus as a diagnostic antigen in ELISA to differentiate infected from vaccinated cattle. Archives of Virology 1, 2021–2033.

Ding, Y.Z., Zhou, J.H., Ma, L.N., Qi, Y.N., Wei, G., Zhang, J., Zhang, Y.G., 2014. A reverse transcription loop-mediated isothermal amplification assay to rapidly diagnose foot-and-mouth disease virus C. Korean Journal of Thoracic and Cardiovascular Surgery 47, 423–426. https://doi.org/10.5090/kjtcs.2014.47.4.423

Doel, T.R., 2005. Natural and vaccine induced immunity to FMD. Current Topics in Microbiology and Immunology 288, 103–131. https://doi.org/10.1007/3-540-27109-0_5

Doel, T.R., 2003. FMD vaccines. Virus Research 91, 81–99.

Doel, T.R., 1996. Natural and vaccine-induced immunity to foot and mouth disease: the prospects for improved vaccines. Revue scientifique et technique International Office of Epizootics 15, 883–911. https://doi.org/10.20506/rst.15.3.955

Doherty, M.L., Bassett, H.F., Quinn, P.J., Davis, W.C., Kelly, A.P., Monaghan, M.L., 1996. A sequential study of the bovine tuberculin reaction. Immunology 87, 9–14.

Domingo, E., Escarmís, C., Baranowski, E., Ruiz-Jarabo, C.M., Carrillo, E., Núñez, J.I., Sobrino,

F., 2003. Evolution of foot-and-mouth disease virus. Virus Research 91, 47–63.

Domingo, E., Holland, J.J., 1997. RNA virus mutations and fitness for survival. Annual Review of Microbiology 51, 151–178. https://doi.org/10.1146/annurev.micro.51.1.151

Donaldson, A., 2004. Clinical signs of foot and mouth disease, in: Sobrino, F.D.E. (Ed.), Foot and Mouth Disease Current Perspectives. CRC Press, Boca Raton, pp. 93–102. https://doi.org/https://doi.org/10.1201/9780429125614

Donaldson, A., 1999. Airborne spread of foot-and-mouth disease. Microbiology Today 26, 118–119. https://doi.org/10.1071/AP97011

Donaldson, A.I., 1999. Foot-and-mouth-disease in western North Africa: an analysis of the risk for Europe, in: Proceedings of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Maisons-Alfort, France 29 September to 1 October 1999, Appendix 5, P45 FAO, Rome. FAO, Rome, Rome, p. 45.

Dovie, D.B.K., Shackleton, C.M., Witkowski, E.T.F., 2006. Valuation of communal area livestock benefits, rural livelihoods and related policy issues. Land Use Policy 23, 260–271. https://doi.org/10.1016/j.landusepol.2004.08.004

Drake, J.W., Holland, J.J., 1999. Mutation rates among RNA viruses. Proceedings of the National Academy of Sciences of the United States of America 96, 13910–13913.

Dube, C., Ribble, C., Kelton, D., McNab, B., 2009. A review of network analysis terminology and its application to foot-and-mouth disease modelling and policy development. Transboundary and Emerging Diseases 56, 73–85.

Dubé, C., Ribble, C., Kelton, D., McNab, B., 2011. Introduction to network analysis and its implications for animal disease modelling. Revue Scientifique et Technique (International Office of Epizootics) 30, 425–36.

Dubé, C., Ribble, C., Kelton, D., McNab, B., 2008. Comparing network analysis measures to determine potential epidemic size of highly contagious exotic diseases in fragmented monthly networks of dairy cattle movements in Ontario, Canada. Transboundary and Emerging Diseases 55, 382–392.

Dyason, E., 2010. Summary of foot-and-mouth disease outbreaks reported in and around the Kruger National Park, South Africa, between 1970 and 2009. Journal of the South African Veterinary Association 81, 201–206.

Eblé, P.L., Bouma, A., De Bruin, M.G.M., Van Hemert-Kluitenberg, F., Van Oirschot, J.T., Dekker, A., 2004. Vaccination of pigs two weeks before infection significantly reduces transmission of foot-and-mouth disease virus. Vaccine 22, 1372–1378. https://doi.org/ 10.1016/j.vaccine.2003.11.003

Ehizibolo, D.O., Fish, I.H., Brito, B., Bertram, M.R., Ardo, A., Ularamu, H.G., Lazarus, D.D., Wungak, Y.S., Nwosuh, C.I., Smoliga, G.R., Hartwig, E.J., Pauszek, S.J., Dickmu, S., Abdoulkadiri, S., Arzt, J., 2019. Characterization of transboundary foot-and-mouth disease viruses in Nigeria and Cameroon during 2016. Transboundary and Emerging Diseases. https://doi.org/10.1111/tbed.13461

Ehizibolo, D.O., Haegeman, A., De Vleeschauwer, A.R., Umoh, J.U., Kazeem, H.M., Okolocha, E.C., Van Borm, S., De Clercq, K., 2017. Detection and Molecular Characterization of Foot and Mouth Disease Viruses from Outbreaks in Some States of Northern Nigeria 2013–2015.

Transboundary and Emerging Diseases 64, 1979–1990. https://doi.org/10.1111/tbed.12602

Ellis-Iversen, J., Smith, R.P., Gibbens, J.C., Sharpe, C.E., Dominguez, M., Cook, A.J.C., 2011. Papers: Risk factors for transmission of foot-and-mouth disease during an outbreak in southern England in 2007. Veterinary Record 168, 128. https://doi.org/10.1136/vr.c6364

Elnaker, Y.F., Sayed-Ahmed, M., El-Beskawy, M., El-Sawalhy, A.A., Wabacha, J.K., 2013. Seroprevalence of FMD in cattle, sheep and goats in Somaliland. Bulletin of Animal Health and Production in Africa 60, 383–392.

Elnekave, E., Shilo, H., Gelman, B., Klement, E., 2015. The longevity of anti NSP antibodies and the sensitivity of a 3ABC ELISA - A 3 years follow up of repeatedly vaccinated dairy cattle infected by foot and mouth disease virus. Veterinary Microbiology 178, 14–18. https://doi.org/10.1016/j.vetmic.2015.04.003

Elnekave, E., Van Maanen, C., Shilo, H., Gelman, B., Storm, N., Berdenstain, S., Berke, O., Klement, E., 2016. Prevalence and risk factors for foot and mouth disease infection in small ruminants in Israel. Preventive Veterinary Medicine 125, 82–88. https://doi.org/10.1016/j.prevetmed.2015.12.019

Els, H., 1996. Socio-economics of the animal husbandry in the rural communal areas of South Africa, in: McCrindle, C. M. E. & Krecek, E. C. (Ed.), Socio-Economics of Veterinary Research and Training: A Forum. MEDUNSA, Pretoria.

Eschbaumer, M., Stenfeldt, C., Rekant, S.I., Pacheco, J.M., Hartwig, E.J., Smoliga, G.R., Kenney, M.A., Golde, W.T., Rodriguez, L.L., Arzt, J., 2016. Systemic immune response and virus persistence after foot-and-mouth disease virus infection of naïve cattle and cattle vaccinated with a homologous adenovirus-vectored vaccine. BMC Veterinary Research 12.

https://doi.org/10.1186/s12917-016-0838-x

FAO, 2016. Foot and mouth disease vaccination monitoring Guidelines. FAO, Rome.

FAO, 2011. The Progressive Control Pathway for FMD Control (PCP-FMD) Principles, Stage Descriptions and Standards.

Fasina, F.O., Connell, D.R., Talabi, O.A., Lazarus, D.D., Adeleke, G.A., Olusanya, T.P., Hernandez, J.A., 2013. Foot-and-mouth disease virus strains and examination of exposure factors associated with seropositivity of cattle herds in Nigeria during 2007-2009. Preventive Veterinary Medicine 109, 334–42. https://doi.org/10.1016/j.prevetmed.2012.10.004

Fasina FO, Mokoele JM, Spencer BT, van Leengoed LAML, Bevis Y, B.I., 2015. Spatio-temporal patterns and movement analysis of pigs from smallholder farms and implications for African swine fever spread , Limpopo province , South Africa. Onderstepoort Journal of Veterinary Research 82, 1–11. https://doi.org/10.4102/ojvr.v82i1.795

Ferguson, N.M., Donnelly, C.A., Anderson, R.M., 2001. Transmission intensity and impact of control policies on the foot and mouth epidemic in Great Britain. Nature 413, 542–548. https://doi.org/10.1038/35097116

Ferris, N.P., King, D.P., Reid, S.M., Shaw, A.E., Hutchings, G.., Ferris, N.P., King, D.P., Reid, S.M., Shaw, A.E., Hutchings, G.H., 2006. Comparisons of original laboratory results and retrospective analysis by real-time reverse transcriptase-PCR of virological samples collected from confirmed cases of foot-and-mouth disease in the UK in 2001. Veterinary Record 159, 373–378.

Ferris, N.P., Clavijo, A., Yang, M., Velazquez-Salinas, L., Nordengrahn, A., Hutchings, G.H.,

Kristersson, T., Merza, M., 2012. Development and laboratory evaluation of two lateral flow devices for the detection of vesicular stomatitis virus in clinical samples. Journal of Virological Methods 180, 96–100. https://doi.org/10.1016/j.jviromet.2011.12.010

Ferris, N.P., Dawson, M., 1988. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases. Veterinary Microbiology 16, 201–209.

Ferris, N.P., Nordengrahn, A., Hutchings, G.H., Paton, D.J., Kristersson, T., Brocchi, E., Grazioli, S., Merza, M., 2010. Development and laboratory validation of a lateral flow device for the detection of serotype SAT 2 foot-and-mouth disease viruses in clinical samples. Journal of Virological Methods 163, 474–476.

Ferris, N.P., Nordengrahn, A., Hutchings, G.H., Reid, S.M., King, D.P., Ebert, K., Paton, D.J., Kristersson, T., Brocchi, E., Grazioli, S., Merza, M., 2009. Development and laboratory validation of a lateral flow device for the detection of foot-and-mouth disease virus in clinical samples. Journal of Virological Methods 155, 10–17.

Fowler, V.L., Bankowski, B.M., Armson, B., Di Nardo, A., Valdazo-Gonzalez, B., Reid, S.M., Barnett, P. V., Wadsworth, J., Ferris, N.P., Mioulet, V., King, D.P., 2014. Recovery of viral RNA and infectious foot-and-mouth disease virus from positive lateral-flow devices. PLoS ONE 9. https://doi.org/10.1371/journal.pone.0109322

Francis, M.J., Black, L., 1983. Antibody response in pig nasal fluid and serum following foot-and-mouth disease infection or vaccination. Journal of Hygiene 91, 329–334. https://doi.org/10.1017/S0022172400060344

Frössling, J., Ohlson, A., Björkman, C., Håkansson, N., Nöremark, M., 2012. Application of

network analysis parameters in risk-based surveillance - Examples based on cattle trade data and bovine infections in Sweden. Preventive Veterinary Medicine 105, 202–208.

Fukai, K., Onozato, H., Kitano, R., Yamazoe, R., Morioka, K., Yamada, M., Ohashi, S., Yoshida, K., Kanno, T., 2013. Availability of a fetal goat tongue cell line ZZ-R 127 for isolation of Foot-and-mouth disease virus (FMDV) from clinical samples collected from animals experimentally infected with FMDV. Journal of Veterinary Diagnostic Investigation 25, 770–774. https://doi.org/10.1177/1040638713508400

Gao, M., Zhang, R., Li, M., Li, S., Cao, Y., Ma, B., Wang, J., 2012. An ELISA based on the repeated foot-and-mouth disease virus 3B epitope peptide can distinguish infected and vaccinated cattle. Applied Microbiology and Biotechnology 93, 1271–1279. https://doi.org/10.1007/s00253-011-3815-0

Garland, A.J.M., 1999. Vital elements for the successful control of foot-and-mouth disease by vaccination. Vaccine 17, 1760–1766. https://doi.org/10.1016/S0264-410X(98)00443-5

Germain, R.N., 1994. MHC-dependent antigen processing and peptide presentation: Providing ligands for T lymphocyte activation. Cell 76, 287–299. https://doi.org/10.1016/0092-8674(94)90336-0

Gibbens, J.C., Sharpe, C.E., Wilesmith, J.W., Mansley, L.M., Michalopoulou, E., Ryan, J.B.M., Hudson, M., 2001. Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: The first five months. Veterinary Record 149, 729–743. https://doi.org/10.1136/vr.149.24.729

Gibson, C.F., Donaldson, A.I., 1986. Exposure of sheep to natural aerosols of foot-and-mouth disease virus. Research in Veterinary Science 41, 45–49. https://doi.org/10.1016/s0034-

5288(18)30570-8

Gleeson, L.J., Samuel, A.R., Knowles, N.J., 2003. Epidemiology of foot-and-mouth disease in Southeast Asia, in: Dodet, B. & Vicari, M. (Ed.), Foot and Mouth Disease: Control Strategies. Elsevier, Paris, pp. 85–102.

Golde, W.T., Nfon, C.K., Toka, F.N., 2008. Immune evasion during foot-and-mouth disease virus infection of swine. Immunological Reviews 225, 85–95. https://doi.org/10.1111/j.1600-065X.2008.00672.x

Green, D.M., Kiss, I.Z., Mitchell, A.P., Kao, R.R., 2008. Estimates for local and movement-based transmission of bovine tuberculosis in British cattle. Proceedings of the Royal Society B: Biological Sciences 275, 1001–1005. https://doi.org/10.1098/rspb.2007.1601

Grubman, M.J., Baxt, B., 2004. Foot-and-mouth disease. Clinical Microbiology Reviews 17, 465–493.

Guzman, Efrain., Sally, Price., Hannah, Poulsom., Jayne, H., 2012. Bovine gamma delta T cells: Cells with multiple functions and important roles in immunity. Veterinary Immunology and Immunopathology 148, 161–167.

Habiela, M., Alamin, M.A.G., Raoui, Y.A., Yahia, H.A.H., Mohammed, A., Alamin, G., Raouf, Y.A., Ali, Y.H., Alamin, M.A.G., Raoui, Y.A., Yahia, H.A.H., Mohammed, A., Alamin, G., Raouf, Y.A., Ali, Y.H., 2010. Epizootiological study of foot and mouth disease in the Sudan: the situation after two decades. Veterinarski arhiv 80, 11–26.

Hall, T.A., 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symposium Series 41, 95–98.

Hamblin C, Kitching RP, Donaldson AI, Crowther JR, B.I., 1987. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. Epidemiology and Infection 99, 733–744.

Hamblin, C., Barnett, I.T., Hedger, R.S., 1986. A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. I. Development and method of ELISA. Journal of immunological methods 93, 115–21.

Han, L., Xin, X., Wang, H., Li, J., Hao, Y., Wang, M., Zheng, C., Shen, C., 2018. Cellular response to persistent foot-and-mouth disease virus infection is linked to specific types of alterations in the host cell transcriptome. Scientific Reports 8, 1–13. https://doi.org/10.1038/s41598-018-23478-0

Hargreaves, S.K., Foggin, C.M., Anderson, E.C., Bastos, A.D.S., Thomson, G.R., Ferris, N.P., Knowles, N.J., 2004. An investigation into the source and spread of foot and mouth disease virus from a wildlife conservancy in Zimbabwe. Revue Scientifique et Technique (International Office of Epizootics) 23, 783–790.

Haydon, D.T., Kao, R.R., Kitching, R.P., 2004. The UK foot-and-mouth disease outbreak - The aftermath. Nature Reviews Microbiology 2, 675–681. https://doi.org/10.1038/nrmicro960

Hedger RS, F.A. and W.M., 1973. Foot-and-mouth disease in East African buffalo. Bulletin of Epizootic Disease of Africa 21, 99–101.

Hollister, J.R., Vagnozzi, A., Knowles, N.J., Rieder, E., 2008. Molecular and phylogenetic analyses of bovine rhinovirus type 2 shows it is closely related to foot-and-mouth disease virus. Virology 373, 411–425. https://doi.org/10.1016/j.virol.2007.12.019

Horsington, J., Nfon, C., Gonzales, J.L., Singanallur, N., Bittner, H., Vosloo, W., 2018. Protection in sheep against heterologous challenge with serotype Asia-1 foot-and-mouth disease virus using high potency vaccine. Vaccine 36, 6095–6102. https://doi.org/10.1016/j.vaccine.2018.08.073

Horsington, J., Zhang, Z., Bittner, H., Hole, K., Singanallur, N.B., Alexandersen, S., Vosloo, W., 2015. Early protection in sheep against intratypic heterologous challenge with serotype O foot-and-mouth disease virus using high-potency, emergency vaccine. Vaccine 33. https://doi.org/10.1016/j.vaccine.2014.11.043

House, C. and House, J.A., 1989. Evaluation of techniques to demonstrate foot-and-mouth disease virus in bovine tongue epithelium: Comparison of the sensitivity of cattle, mice, primary cell cultures, cryopreserved cell cultures and established cell lines. Veterinary Microbiology 20, 99–109.

Hughes GJ, Mioulet V, Kitching RP, Woolhouse MEJ, Alexandersen S, D.A., Hughes, G. J., Mioulet, V., Kitching, R. P., Woolhouse, M. E., Alexandersen, S., Donaldson, A.I., 2002. Foot-and-mouth disease virus infection in sheep: implications for diagnosis and control. Veterinary Record 150, 724–727.

Hunter, P., 1998. Vaccination as a means of control of foot-and-mouth disease in sub-saharan Africa. Vaccine 16, 261–264.

Hunter, P., 1996. The performance of southern African territories serotypes of foot and mouth disease antigen in oil-adjuvanted vaccines. Revue scientifique et technique (International Office of Epizootics) 15, 913–22.

Hutcheon, D., 1892. Annual Report of the Colonial Veterinary Surgeon.

Hyera, J.M.K., Letshwenyo, M., Monyame, K.B., Thobokwe, G., Pilane, a R., Mapitse, N., Baipoledi, E.K., 2006. A serological survey for antibodies to foot-and-mouth disease virus in indigenous Tswana goats and sheep in Kasane, Maun and Shakawe districts in northwestern Botswana. The Onderstepoort Journal of Veterinary Research 73, 143–147.

Ivanovic, S., Pavlovic, I., Pisinov, B., 2016. The quality of goat meat and it's impact on human health. Biotechnology in Animal HusbandryBiotehnologija u stocarstvu 32. https://doi.org/10.2298/bah1602111i

Jackson, T., Mould, A.P., Sheppard, D., King, A.M.Q., 2002. Integrin αvβ1 Is a Receptor for Foot-and-Mouth Disease Virus. Journal of Virology 76, 935–941. https://doi.org/10.1128/jvi.76.3.935-941.2002

Jamal, S.M., Belsham, G.J., 2013. Foot-and-mouth disease: Past, present and future. Veterinary Research 44. https://doi.org/10.1186/1297-9716-44-116

Jaworski, J.P., Compaired, D., Trotta, M., Perez, M., Trono, K., Fondevila, N., 2011. Validation of an r3AB1-FMDV-NSP ELISA to distinguish between cattle infected and vaccinated with foot-and-mouth disease virus. Journal of Virological Methods 178, 191–200. https://doi.org/10.1016/j.jviromet.2011.09.011

Jo, H.E., You, S.H., Choi, J.H., Ko, M.K., Shin, S.H., Song, J., Jo, H., Lee, M.J., Kim, S.M., Kim, B., Park, J.H., 2019. Evaluation of novel inactivated vaccines for the SAT 1, SAT 2 and SAT 3 serotypes of foot-and-mouth disease in pigs. Virology Journal 16. https://doi.org/10.1186/s12985-019-1262-1

Jori, F., Brahmbhatt, D., Fosgate, G.T., Thompson, P.N., Budke, C., Ward, M.P., Ferguson, K., Gummow, B., 2011. A questionnaire-based evaluation of the veterinary cordon fence

separating wildlife and livestock along the boundary of the Kruger National Park, South Africa. Preventive Veterinary Medicine 100, 210–220. https://doi.org/10.1016/j.prevetmed.2011.03.015

Jori, F., Etter, E., 2016. Transmission of foot and mouth disease at the wildlife/livestock interface of the Kruger National Park, South Africa: can the risk be mitigated? Preventive Veterinary Medicine 126, 19–29. https://doi.org/10.1016/j.prevetmed.2016.01.016

Jori, F., Vosloo, W., Du Plessis, B., Bengis, R., Brahmbhatt, D., Gummow, B., Thomson, G.R., 2009. A qualitative risk assessment of factors contributing to foot and mouth disease outbreaks in cattle along the western boundary of the Kruger National Park. OIE Revue Scientifique et Technique 28, 917–931. https://doi.org/10.20506/rst.28.3.1932

Juleff, N., Windsor, M., Lefevre, E. a, Gubbins, S., Hamblin, P., Reid, E., McLaughlin, K., Beverley, P.C.L., Morrison, I.W., Charleston, B., 2009. Foot-and-mouth disease virus can induce a specific and rapid CD4+ T-cell-independent neutralizing and isotype class-switched antibody response in naïve cattle. Journal of Virology 83, 3626–3636.

Kao, R.R., Danon, L., Green, D.M., Kiss, I.Z., 2006. Demographic structure and pathogen dynamics on the network of livestock movements in Great Britain. Proceedings of the Royal Society B: Biological Sciences 273, 1999–2007. https://doi.org/10.1098/rspb.2006.3505

Kasanga, C.J., Yamazaki, W., Mioulet, V., King, D.P., Mulumba, M., Ranga, E., Deve, J., Mundia, C., Chikungwa, P., Joao, L., Wambura, P.N., Rweyemamu, M.M., 2014. Rapid, sensitive and effective diagnostic tools for foot-and-mouth disease virus in Africa. Onderstepoort Journal of Veterinary Research 81, 1–5. https://doi.org/10.4102/ojvr.v81i2.727

Keeling, M.J., Woolhouse, M.E.J., May, R.M., Davies, G., Grenfell, B.T., 2003. Modelling

vaccination strategies against foot-and-mouth disease. Nature 421, 136–142. https://doi.org/10.1038/nature01343

Khengwa, C., Jongchansittoe, P., Sedwisai, P., Wiratsudakul, A., 2017. A traditional cattle trade network in Tak province, Thailand and its potential in the spread of infectious diseases. Animal Production Science 57, 152–160. https://doi.org/10.1071/AN15043

Kinsley, A.C., Patterson, G., VanderWaal, K.L., Craft, M.E., Perez, A.M., 2016. Parameter values for epidemiological models of foot-and-mouth disease in Swine. Frontiers in Veterinary Science 3, 44. https://doi.org/10.3389/fvets.2016.00044

Kiss, I.Z., Green, D.M., Kao, R.R., 2008. The effect of network mixing patterns on epidemic dynamics and the efficacy of disease contact tracing. Journal of the Royal Society Interface 5, 791–799. https://doi.org/10.1098/rsif.2007.1272

Kiss, I.Z., Green, D.M., Kao, R.R., 2006. The network of sheep movements within Great Britain: Network properties and their implications for infectious disease spread. Journal of the Royal Society, Interface / the Royal Society 3, 669–677.

Kitching, R.P., 2002. Clinical variation in foot and mouth disease: cattle. Revue scientifique et technique International Office of Epizootics 21, 499–504.

Kitching, R.P., 1998. A recent history of foot-and-mouth disease. Journal of Comparative Pathology 118, 89–108. https://doi.org/10.1016/S0021-9975(98)80002-9

Kitching, R.P., Hughes, G.J., 2002. Clinical variation in foot and mouth disease: sheep and goats. Revue scientifique et technique (International Office of Epizootics) 21, 505–512.

Knight-Jones, T.J.D., Bulut, A.N., Gubbins, S., Stärk, K.D.C., Pfeiffer, D.U., Sumption, K.J.,

Paton, D.J., 2014. Retrospective evaluation of foot-and-mouth disease vaccine effectiveness in Turkey. Vaccine 32, 1848–1855.

Knight-Jones, T.J.D., Robinson, L., Charleston, B., Rodriguez, L.L., Gay, C.G., Sumption, K.J., Vosloo, W., 2016. Global Foot-and-Mouth Disease Research Update and Gap Analysis: 4 - Diagnostics. Transboundary and Emerging Diseases 63, 42–48. https://doi.org/10.1111/tbed.12523

Knight-Jones, T.J.D., Rushton, J., 2013. The economic impacts of foot and mouth disease - What are they, how big are they and where do they occur? Preventive Veterinary Medicine 112, 161–173. https://doi.org/10.1016/j.prevetmed.2013.07.013

Knowles, N.J., Bachanek-Bankowska, K., Wadsworth, J., Mioulet, V., Valdazo-González, B., Eldaghayes, I.M., Dayhum, A.S., Kammon, A.M., Sharif, M.A., Waight, S., Shamia, A.M., Tenzin, S., Wernery, U., Grazioli, S., Brocchi, E., Subramaniam, S., Pattnaik, B., King, D.P., 2016. Outbreaks of Foot-and-Mouth Disease in Libya and Saudi Arabia During 2013 Due to an Exotic O/ME-SA/Ind-2001 Lineage Virus. Transboundary and emerging diseases 63, e431–e435. https://doi.org/10.1111/tbed.12299

Knowles, N.J., Samuel, A.R., 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Research 91, 65–80. https://doi.org/10.1016/S0168-1702(02)00260-5

Kraemer, M.U.G., Yang, C.-H., Gutierrez, B., Wu, C.-H., Klein, B., Pigott, D.M., du Plessis, L., Faria, N.R., Li, R., Hanage, W.P., Brownstein, J.S., Layan, M., Vespignani, A., Tian, H., Dye, C., Pybus, O.G., Scarpino, S. V, 2020. The effect of human mobility and control measures on the COVID-19 epidemic in China. Science eabb4218. https://doi.org/10.1126/science.abb4218

Krystynak, R.H., Charlebois, P.A., 1987. The Potential Economic Impact of an Outbreak of Foot-and-Mouth Disease in Canada. The Canadian veterinary journal. La revue veterinaire canadienne 28, 523–527.

LaRocco, M., Krug, P.W., Kramer, E., Ahmed, Z., Pacheco, J.M., Duque, H., Baxt, B., Rodriguez, L.L., 2013. Erratum: A continuous bovine kidney cell line constitutively expressing bovine αvβ6 integrin has increased susceptibility to foot-and-mouth disease virus. Journal of Clinical Microbiology 51, 1714–1720. https://doi.org/10.1128/JCM.03220-14

Lavoria, M. ángeles, Di-Giacomo, S., Bucafusco, D., Franco-Mahecha, O.L., Pérez-Filgueira, D.M., Capozzo, A.V., 2012. Avidity and subtyping of specific antibodies applied to the indirect assessment of heterologous protection against Foot-and-Mouth Disease Virus in cattle. Vaccine 30, 6845–6850. https://doi.org/10.1016/j.vaccine.2012.09.011

Lazarus, D. D., Schielen, W. J. G., Wungak, Y., Kwange, D., Fasina, F.O., 2012. Sero-epidemiology of foot-and-mouth disease in some Border States of Nigeria. African Journal of Microbiology Research 6, 1756–1761. https://doi.org/10.5897/ajmr11.1026

Lazarus, D.D., Fosgate, G.T., van Schalkwyk, O.L., Burroughs, R.E.J., Heath, L., Maree, F.F., Blignaut, B., Reininghaus, B., Mpehle, A., Rikhotso, O., Thomson, G.R., 2017. Serological evidence of vaccination and perceptions concerning Foot-and-Mouth Disease control in cattle at the wildlife-livestock interface of the Kruger National Park, South Africa. Preventive Veterinary Medicine 147, 17–25. https://doi.org/10.1016/j.prevetmed.2017.08.016

Lazarus, D.D., Mutowembwa, P.B., Sirdar, M.M., Rametse, T.M., Heath, L., Opperman, P.A., Burroughs, R.E.J., Fosgate, G.T., 2019. Clinical presentation of FMD virus SAT1 infections in experimentally challenged indigenous South African goats. Small Ruminant Research 180,

15–20. https://doi.org/10.1016/J.SMALLRUMRES.2019.09.014

Lazarus, D.D., van Schalkwyk, O.L., Burroughs, R.E.J., Mpehle, A., Reininghaus, B., Rikhotso, O., Heath, L., Maree, F.F., Blignaut, B., Fosgate, G.T., 2018. Serological responses of cattle inoculated with inactivated trivalent foot-and-mouth disease vaccine at the wildlife-livestock interface of the Kruger National Park, South Africa. Preventive Veterinary Medicine 158, 89–96. https://doi.org/10.1016/j.prevetmed.2018.08.003

Leforban, Y., Gerbier, G., 2003. Recent history and epidemiology of foot-and-mouth disease in Europe, in: Dodet, B. & Vivari, M. (Ed.), Foot and Mouth Disease: Control Strategies. Elsevier, Paris, pp. 153–171.

Legesse, Y., Asfaw, Y., Sahle, M., Ayelet, G., Jenberie, S., Negussie, H., 2013. First confirmation of foot and mouth disease virus serotype SAT-1 in cattle and small ruminants in Ethiopia in 2007/08. Tropical Animal Health and Production 45, 1265–1267.

Li, Y., Swabey, K.G., Gibson, D., Keel, P.J., Hamblin, P., Wilsden, G., Corteyn, M., Ferris, N.P., 2012. Evaluation of the solid phase competition ELISA for detecting antibodies against the six foot-and-mouth disease virus non-O serotypes. Journal of Virological Methods 183, 125–131. https://doi.org/10.1016/j.jviromet.2012.04.002

Lin, T., Shao, J.J., Du, J.Z., Cong, G.Z., Gao, S.D., Chang, H., 2011. Development of a serotype colloidal gold strip using monoclonal antibody for rapid detection type Asia1 foot-and-mouth disease. Virology Journal 8, 418. https://doi.org/10.1186/1743-422X-8-418

Littlejohn, A.I., 1970. Foot-and-mouth disease in sheep. State Veterinary Journal 25, 85–96.

Ludi, Anna, Valerie Mioulet, N.J.K. and D.P.K., 2017. Laboratory diagnostic methods to support

the surveillance and control of foot-and-mouth disease, in: Sobrino, F., Domingo, E. (Ed.), Current Research and Emerging Trends. EBSCO Publishers, pp. 275–285.

Lyons N, Lyoo Y, King D, P.D., 2016. Challenges of generating and maintaining protective vaccine-induced immune responses for foot-and-mouth disease virus in pigs. Frontiers in Veterinary Science 3, 102.

Mackay, D.K.J., Bulut, A.N., Rendle, T., Davidson, F., Ferris, N.P., 2001. A solid-phase competition ELISA for measuring antibody to foot-and-mouth disease virus. Journal of Virological Methods 97, 33–48.

Maclachlan, N., Dubori, E.J., 2011. Antiviral Immunity and Prophylaxis, in: Fenner's Veterinary Virology. Academic Press Inc, pp. 75–86.

Madhanmohan, M., Nagendrakumar, S.B., Kumar, R., Anilkumar, J., Manikumar, K., Yuvaraj, S., Srinivasan, V.A., 2012. Clinical protection, sub-clinical infection and persistence following vaccination with extinction payloads of O1 Manisa foot-and-mouth disease monovalent vaccine and challenge in goats and comparison with sheep. Research in Veterinary Science 93, 1050–1059.

Madhanmohan, M., Nagendrakumar, S.B., Manikumar, K., Yuvaraj, S., Parida, S., Srinivasan, V.A., 2013. Development and evaluation of a real-time reverse transcription-loop-mediated isothermal amplification assay for rapid serotyping of foot-and-mouth disease virus. Journal of Virological Methods 187, 195–202. https://doi.org/10.1016/j.jviromet.2012.08.015

Madhanmohan, M., Nagendrakumar, S.B., Narasu, M.L., Srinivasan, V.A., 2010a. Effect of FMD vaccine antigen payload on protection, sub-clinical infection and persistence following needle challenge in sheep. Comparative Immunology, Microbiology and Infectious Diseases 33, e7–

e13. https://doi.org/10.1016/j.cimid.2009.10.001

Madhanmohan, M., Nagendrakumar, S.B., Santhakumar, P., Thiagarajan, D., Narasu, M.L., Srinivasan, V.A., 2011. Immune Response in Goats to Different Payloads of FMDV Monovalent Vaccine: Protection Against Virulent Challenge and Development of Carrier Status. Indian Journal of Microbiology 51, 88–93. https://doi.org/10.1007/s12088-011-0101-x

Madhanmohan, M., Nagendrakumar, S.B., Srinivasan, V.A., 2010b. Protection against direct in-contact challenge following foot-and-mouth disease vaccination in sheep and goats: The effect on virus excretion and carrier status. Veterinary Research Communications 34, 285–299.

Madhanmohan, M., Tresamol, P. V., Saseendranath, M.R., 2009. Immune response in goats to two commercial foot-and-mouth disease vaccines and the assessment of maternal immunity in their kids. Transboundary and Emerging Diseases 56, 49–53. https://doi.org/10.1111/j.1865-1682.2008.01056.x

Maree, F.F., Nsamba, P., Mutowembwa, P., Rotherham, L.S., Esterhuysen, J., Scott, K., 2015. Intra-serotype SAT2 chimeric foot-and-mouth disease vaccine protects cattle against FMDV challenge. Vaccine 33, 2909–2916. https://doi.org/10.1016/j.vaccine.2015.04.058

Martinez-Lopez, B., Perez, A.M., Sanchez-Vizcano, J.M., 2009. Social network analysis. Review of general concepts and use in preventive veterinary medicine. Transboundary and Emerging Diseases 56, 109–120.

Mason, P.W., Grubman, M.J., Baxt, B., 2003. Molecular basis of pathogenesis of FMDV. Virus Research 91, 9–32. https://doi.org/10.1016/S0168-1702(02)00257-5

Mason, P.W., Piccone, M.E., McKenna, T.S.C., Chinsangaram, J., Grubman, M.J., 1997. Evaluation of a live-attenuated foot-and-mouth disease virus as a vaccine candidate. Virology 227, 96–102. https://doi.org/10.1006/viro.1996.8309

Mateu, M.G., Da Silva, J.L., Rocha, E., De Brum, D.L., Alonso, A., Enjuanes, L., Domingo, E., Barahona, H., 1988. Extensive antigenic heterogeneity of foot-and-mouth disease virus of serotype C. Virology 167, 113–124.

Mavridis, G.L., 2018. Clinical signs of foot and Mouth disease in flick of Saanen goats in the village of N. Sofiko. Journal of the Hellenic Veterinary Medical Society 51, 69. https://doi.org/10.12681/jhvms.15660

McCullough, K.C., Bruckner, L., Schaffner, R., Fraefel, W., Müller, H.K., Kihm, U., 1992a. Relationship between the anti-FMD virus antibody reaction as measured by different assays, and protection in vivo against challenge infection. Veterinary Microbiology 30, 99–112.

McCullough, K.C., De Simone, F., Brocchi, E., Capucci, L., Crowther, J.R., Kihm, U., 1992b. Protective immune response against foot-and-mouth disease. Journal of Virology 66, 1835–1840. https://doi.org/10.1128/jvi.66.4.1835-1840.1992

McVicar, J.W. and Sutmoller, P., 1968. Sheep and goats as foot-and-mouth disease carriers, in: US Livestock Sanitary Association Proceedings. pp. 72:400-406.

McVicar, J.W., Sutmoller, P., 1972. Experimental foot-and-mouth disease in sheep and goats: An epizootiological model. Archiv für die gesamte Virusforschung 38, 85–96. https://doi.org/10.1007/BF01241358

Mdladla, K., Dzomba, E.F., Huson, H.J., Muchadeyi, F.C., 2016. Population genomic structure

and linkage disequilibrium analysis of South African goat breeds using genome-wide SNP data. Animal genetics 47, 471–482. https://doi.org/10.1111/age.12442

Megersa, B., Beyene, B., Abunna, F., Regassa, A., Amenu, K., Rufael, T., 2009. Risk factors for foot and mouth disease seroprevalence in indigenous cattle in Southern Ethiopia: The effect of production system. Tropical Animal Health and Production 41, 891–898. https://doi.org/10.1007/s11250-008-9276-5

Meyer, R.F., Brown, C.C., House, C., House, J.A., Molitor, T.W., 1991. Rapid and sensitive detection of foot-and-mouth disease virus in tissues by enzymatic RNA amplification of the polymerase gene. Journal of Virological Methods 34, 161–172. https://doi.org/10.1016/0166-0934(91)90096-I

Milon, G., Louis, J., 1993. CD8+ T cells and immunity to intracellular pathogens. Parasitology Today 9, 196–197. https://doi.org/10.1016/0169-4758(93)90002-W

Mohana Subramanian, B., Madhanmohan, M., Sriraman, R., Chandrasekhar Reddy, R. V., Yuvaraj, S., Manikumar, K., Rajalakshmi, S., Nagendrakumar, S.B., Rana, S.K., Srinivasan, V.A., 2012. Development of foot-and-mouth disease virus (FMDV) serotype O virus-like-particles (VLPs) vaccine and evaluation of its potency. Antiviral Research 96, 288–295. https://doi.org/10.1016/j.antiviral.2012.09.019

Mohapatra, A.K., Mohapatra, J.K., Pandey, L.K., Sanyal, A., Pattnaik, B., 2014. Diagnostic potential of recombinant nonstructural protein 3B to detect antibodies induced by foot-and-mouth disease virus infection in bovines. Archives of virology 159, 2359–2369. https://doi.org/10.1007/s00705-014-2089-0

Molla, B., Ayelet, G., Asfaw, Y., Jibril, Y., Ganga, G., Gelaye, E., 2010. Epidemiological study

on foot-and-mouth disease in cattle: Seroprevalence and risk factor assessment in south omo zone, south-western Ethiopia. Transboundary and Emerging Diseases 57, 340–347. https://doi.org/10.1111/j.1865-1682.2010.01154.x

Moniwa, M., Clavijo, A., Li, M., Collignon, B., Paul Kitching, R., 2007. Performance of a foot-and-mouth disease virus reverse transcription- polymerase chain reaction with amplification controls between three real-time instruments. Journal of Veterinary Diagnostic Investigation 19, 9–20. https://doi.org/10.1177/104063870701900103

Montiel, N.A., Smoliga, G., Arzt, J., 2013. Time-dependent biodistribution and transgene expression of a recombinant human adenovirus serotype 5-luciferase vector as a surrogate for rAd5-FMDV vaccines in cattle. Veterinary Immunology and Immunopathology 151, 37–48. https://doi.org/10.1016/j.vetimm.2012.10.003

Moonen, P., Boonstra, J., Honing, R.H.- Van Der, Leendertse, C.B.-, Jacobs, L., Dekker, A., Hakze- Van Der Honing, R., Boonstra- Leendertse, C., Jacobs, L., Dekker, A., 2003. Validation of a LightCycler-based reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus. Journal of Virological Methods 113, 35–41. https://doi.org/10.1016/S0166-0934(03)00220-9

Mouton, L., Dekker, A., Bleijenberg, M., Blanchet, M., Coco-Martin, J., Hudelet, P., Goutebroze, S., 2018. A foot-and-mouth disease SAT2 vaccine protects swine against experimental challenge with a homologous virus strain, irrespective of mild pathogenicity in this species. Vaccine 36, 2020–2024. https://doi.org/10.1016/j.vaccine.2018.02.096

Murphy, K., Travers, P and Walport, M., 2008. Innate immunity, in: Janeway's Immunobiology. pp. 39–108.

Musser, J.M.B., 2004. A practitioner's primer on foot-and-mouth disease. Journal of the American Veterinary Medical Association 224, 1261–1268.

Mweu, M.M., Fournié, G., Halasa, T., Toft, N., Nielsen, S.S., 2013. Temporal characterisation of the network of Danish cattle movements and its implication for disease control: 2000-2009. Preventive Veterinary Medicine 110, 379–387. https://doi.org/0.1016/j.prevetmed. 2013.02.015

Natale, F., Giovannini, A., Savini, L., Palma, D., Possenti, L., Fiore, G., Calistri, P., 2009. Network analysis of Italian cattle trade patterns and evaluation of risks for potential disease spread. Preventive Veterinary Medicine 92, 341–350. https://doi.org/10.1016/j.prevetmed. 2009.08.026

Niedbalski, W.Ł.A.W., Sy, A.K.Ę., 2010. Rapid detection and quantification of foot-and-mouth disease virus by a real-time reverse transcription PCR. Veterinary Institute Pulawy 1, 3–7.

Noopataya, S., Thongratsakul, S., Poolkhet, C., 2015. Social network analysis of cattle movement in sukhothai province, Thailand: A study to improve control measurements. Veterinary Medicine International.

Nremark, M., Hkansson, N., Lewerin, S.S., Lindberg, A., Jonsson, A., Nöremark, M., Håkansson, N., Lewerin, S.S., Lindberg, A., Jonsson, A., 2011. Network analysis of cattle and pig movements in Sweden: Measures relevant for disease control and risk based surveillance. Preventive Veterinary Medicine 99, 78–90. https://doi.org/10.1016/j.prevetmed.2010.12.009

Nyaguthii, D.M., Armson, B., Kitala, P.M., Sanz-Bernardo, B., Di Nardo, A., Lyons, N.A., 2019. Knowledge and risk factors for foot-and-mouth disease among small-scale dairy farmers in an endemic setting. Veterinary Research 50. https://doi.org/10.1186/s13567-019-0652-0

Oem, J.K., Kye, S.J., Lee, K.N., Kim, Y.J., Park, J.Y., Park, J.H., Joo, Y.S., Song, H.J., 2005. Development of a Lightcycler-based reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus. Journal of Veterinary Science 6, 207–212.

Oh, Y., Fleming, L., Statham, B., Hamblin, P., Barnett, P., Paton, D.J., Park, J.H., Joo, Y.S., Parida, S., 2012. Interferon Induced by In Vitro Re-Stimulation of CD4+ T-Cells Correlates with In Vivo FMD Vaccine Induced Protection of Cattle against Disease and Persistent Infection. PLoS ONE 7.

OIE-WAHID, 2017. World Animal Health Information Database Interface (WAHID) [WWW Document]. Bushbuckridge FMD Outbreak Report. URL https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=13947 (accessed 4.29.19).

OIE, 2019. OIE Members' official FMD status [WWW Document]. OIE. URL http://www.oie.int/en/animal-health-in-the-world/official-disease-status/fmd/en-fmd-carte/ (accessed 8.5.19).

OIE World Organisation for Animal Health, 2018. Foot and Mouth disease (FMD) [WWW Document]. Animal Health in the World. URL http://www.oie.int/en/animal-health-in-the-world/animal-diseases/Foot-and-mouth-disease/ (accessed 4.23.19).

Olah, M., Pavlovic, Panjevic, D., 1976. Clinical picture and differential diagnosis of foot and mouth disease in sheep and goats. Veterinarski Glasnik 30, 239–245.

Onozato, H., Fukai, K., Kitano, R., Yamazoe, R., Morioka, K., Yamada, M., Ohashi, S., Yoshida, K., Kanno, T., 2014. Experimental infection of cattle and goats with a foot-and-mouth disease virus isolate from the 2010 epidemic in Japan. Archives of Virology 159, 2901–2908. https://doi.org/10.1007/s00705-014-2135-y

Ortiz-Pelaez, A., Pfeiffer, D.U., Soares-Magalhães, R.J., Guitian, F.J., 2006. Use of social network analysis to characterize the pattern of animal movements in the initial phases of the 2001 foot and mouth disease (FMD) epidemic in the UK. Preventive Veterinary Medicine 76, 40–55.

Paiba, G.A., Anderson, J., Paton, D.J., Soldan, A.W., Alexandersen, S., Corteyn, M., Wilsden, G., Hamblin, P., MacKay, D.K.J., Donaldson, A.I., 2004. Validation of a foot-and-mouth disease antibody screening solid-phase competition ELISA (SPCE). Journal of Virological Methods 115, 145–158.

Palm, N.W., Medzhitov, R., 2009. Pattern recognition receptors and control of adaptive immunity. Immunological Reviews 227, 221–233. https://doi.org/10.1111/j.1600-065X.2008.00731.x

Parida, S., 2009. Vaccination against foot-and-mouth disease virus: strategies and effectiveness. Expert Review of Vaccines 8, 347–365.

Parida, S., Cox, S.J., Reid, S.M., Hamblin, P., Barnett, P. V, Inoue, T., Anderson, J., Paton, D.J., 2005. The application of new techniques to the improved detection of persistently infected cattle after vaccination and contact exposure to foot-and-mouth disease. Vaccine 23, 5186–5195.

Parida, S., Fleming, L., Oh, Y., Mahapatra, M., Hamblin, P., Gloster, J., Paton, D.J., 2008. Emergency vaccination of sheep against foot-and-mouth disease: Significance and detection of subsequent sub-clinical infection. Vaccine 26, 3469–3479.

Park, J.-H., 2013. Requirements for improved vaccines against foot-and-mouth disease epidemics. Clinical and Experimental Vaccine Research 2, 8. https://doi.org/10.7774/cevr.2013.2.1.8

Park, M.E., Lee, S.Y., Kim, R.H., Ko, M.K., Lee, K.N., Kim, S.M., Kim, B.K., Lee, J.S., Kim, B.,

Park, J.H., 2014. Enhanced immune responses of foot-and-mouth disease vaccine using new oil/gel adjuvant mixtures in pigs and goats. Vaccine 32, 5221–5227. https://doi.org/10.1016/j.vaccine.2014.07.040

Patil, P. K., Bayry, J., Ramakrishna, C., Hugar, B., Misra, L.D., Natarajan, C., 2002. Immune responses of goats against foot-and-mouth disease quadrivalent vaccine: Comparison of double oil emulsion and aluminium hydroxide gel vaccines in eliciting immunity. Vaccine 20, 2781–2789. https://doi.org/10.1016/S0264-410X(02)00184-6

Patil, Prasanna K., Bayry, J., Ramakrishna, C., Hugar, B., Misra, L.D., Prabhudas, K., Natarajan, C., 2002. Immune responses of sheep to quadrivalent double emulsion foot-and-mouth disease vaccines: Rate of development of immunity and variations among other ruminants. Journal of Clinical Microbiology 40, 4367–4371. https://doi.org/10.1128/JCM.40.11.4367-4371.2002

Paton, D.J., de Clercq, K., Greiner, M., Dekker, A., Brocchi, E., Bergmann, I., Sammin, D.J., Gubbins, S., Parida, S., 2006. Application of non-structural protein antibody tests in substantiating freedom from foot-and-mouth disease virus infection after emergency vaccination of cattle. Vaccine 24, 6503–6512. https://doi.org/10.1016/j.vaccine.2006.06.032

Paton, D. J., Ferris, N.P., Hutchings, G.H., Li, Y., Swabey, K., Keel, P., Hamblin, P., King, D.P., Reid, S.M., Ebert, K., Parida, S., Savva, S., Georgiou, K., Kakoyiannis, C., 2009. Investigations into the cause of foot-and-mouth disease virus seropositive small ruminants in Cyprus during 2007. Transboundary and Emerging Diseases 56, 321–328.

Paton, D.J., Gubbins, S., King, D.P., 2018. Understanding the transmission of foot-and-mouth disease virus at different scales. Current Opinion in Virology 28, 85–91.

https://doi.org/10.1016/j.coviro.2017.11.013

Paton, David J., Sumption, K.J., Charleston, B., 2009. Options for control of foot-and-mouth disease: Knowledge, capability and policy. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 2657–2667. https://doi.org/10.1098/rstb.2009.0100

Paton DJ, King DP, Knowles NJ, H.J., 2010. Recent spread of foot-and-mouth disease in the far East. Veterinary Record 166, 569–570.

Pay, T.W., Hingley, P.J., 1987. Correlation of 140S antigen dose with the serum neutralizing antibody response and the level of protection induced in cattle by foot-and-mouth disease vaccines. Vaccine 5, 60–64.

Penrith, M. L and Thomson, G.R., 2012. Analysis of the status of Transboundary Animal Diseases and their control in the SADC Region during the period 2005-2011, Focusing on Five Countries that contribute land to the Kavango Zambezi (KAZA) Transfrontier Conservation Area (TCFA).

Pieters, J., 2000. MHC class II-restricted antigen processing and presentation. Advances in Immunology 75, 159–208. https://doi.org/10.1016/s0065-2776(00)75004-8

Pollock, J.M., Welsh, M.D., 2002. The WC1+ γδ T-cell population in cattle: A possible role in resistance to intracellular infection. Veterinary Immunology and Immunopathology 89, 105–114. https://doi.org/10.1016/S0165-2427(02)00200-3

Poolkhet, C., Kasemsuwan, S., Phiphakhavong, S., Phouangsouvanh, I., Vongxay, K., Shin, M.S., Kalpravidh, W., Hinrichs, J., 2019. Social network analysis for the assessment of pig, cattle and buffalo movement in Xayabouli, Lao PDR. PeerJ 6, e6177. https://doi.org/

10.7717/peerj.6177

Poolkhet, C., Kasemsuwan, S., Seng, S., Keartha, C., Sokmao, C., Shin, M., Kalpravidh, W., Hinrichs, J., 2016. Social network analysis of cattle movement in Kampong Cham, Kampong Speu and Takeo, Cambodia. Acta Tropica 159, 44–49. https://doi.org/10.1016/j.actatropica.2016.03.027

Porta, C., Kotecha, A., Burman, A., Jackson, T., Ren, J., Loureiro, S., Jones, I.M., Fry, E.E., Stuart, D.I., Charleston, B., 2013. Rational Engineering of Recombinant Picornavirus Capsids to Produce Safe, Protective Vaccine Antigen. PLoS Pathogens 9. https://doi.org/10.1371/journal.ppat.1003255

Preston, K.J., Owens, H., Mowat, G.N., 1982. Sources of variations encountered during the selection and production of three strains of FMD virus for the development of vaccine for use in Nigeria. Journal of Biological Standardization 10, 35–45. https://doi.org/10.1016/S0092-1157(82)80046-2

Quan, M., Murphy, C.M., Zhang, Z., Alexandersen, S., 2004. Determinants of early foot-and-mouth disease virus dynamics in pigs. Journal of Comparative Pathology 131, 294–307. https://doi.org/10.1016/j.jcpa.2004.05.002

Reed, L.J., Muench, H., 1937. A simple method of estimating 50% endpoints. The American Journal of Hygiene 27, 493–497. https://doi.org/10.2106/JBJS.K.00472

Reid, E., Charleston, B., 2014. Type I and III interferon production in response to RNA viruses. Journal of Interferon and Cytokine Research 34, 649–658. https://doi.org/10.1089/jir.2014.0066

Reid, S.M., Ebert, K., Bachanek-Bankowska, K., Batten, C., Sanders, A., Wright, C., Shaw, A.E., Ryan, E.D., Hutchings, G.H., Ferris, N.P., Paton, D.J., King, D.P., 2009. Performance of real-time reverse transcription polymerase chain reaction for the detection of Foot-and-mouth disease virus during field outbreaks in the United Kingdom in 2007. Journal of Veterinary Diagnostic Investigation 21, 321–330. https://doi.org/10.1177/104063870902100303

Reid, S.M., Ferris, N.P., Hutchings, G.H., De Clercq, K., Newman, B.J., Knowles, N.J., Samuel, A.R., 2001. Diagnosis of foot-and-mouth disease by RT-PCR: Use of phylogenetic data to evaluate primers for the typing of viral RNA in clinical samples. Archives of Virology 146, 2421–2434. https://doi.org/10.1007/s007050170012

Reid, S.M., Ferris, N.P., Hutchings, G.H., Samuel, A.R., Knowles, N.J., 2000. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. Journal of Virological Methods 89, 167–176. https://doi.org/10.1016/S0166-0934(00)00213-5

Reid SM, Ferris NP, Hutchings GH, Zhang Z, Belsham GJ, A.S., Reid, S.M., Ferris, N.P., Hutchings, G.H., Zhang, Z., Belsham, G.J., Alexandersen, S., 2002. Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay. Journal of Virological Methods 105, 67–80. https://doi.org/10.1016/S0166-0934(02)00081-2

Robinson, L., Knight-Jones, T.J.D., Charleston, B., Rodriguez, L.L., Gay, C.G., Sumption, K.J., Vosloo, W., 2016a. Global Foot-and-Mouth Disease Research Update and Gap Analysis: 3 - Vaccines. Transboundary and Emerging Diseases 63, 30–41. https://doi.org/10.1111/tbed.12521

Robinson, L., Knight-Jones, T.J.D., Charleston, B., Rodriguez, L.L., Gay, C.G., Sumption, K.J.,

Vosloo, W., 2016b. Global Foot-and-Mouth Disease Research Update and Gap Analysis: 6 – Immunology. Transboundary and Emerging Diseases 63, 56–62. https://doi.org/ 10.1111/tbed.12518

Robinson, S.E., Christley, R.M., 2007. Exploring the role of auction markets in cattle movements within Great Britain. Preventive Veterinary Medicine 81, 21–37.

Robinson, S.E., Everett, M.G., Christley, R.M., 2007. Recent network evolution increases the potential for large epidemics in the British cattle population. Journal of the Royal Society Interface 4, 669–674. https://doi.org/10.1098/rsif.2007.0214

Robiolo, B., Grigerat, P.R., Periolot, O.H., Maradeiz, E., Torre, J.L. La, Sekit, C., Bianchil, T., 1995. Assessment of foot and mouth disease vaccine potency by liquid-phase blocking ELISA : a proposal for an alternative to the challenge procedure in Argentina. Vaccine 13, 1346–1352.

Robiolo, B., Seki, C., Fondevilla, N., Grigera, P., Scodeller, E., Periolo, O., Torre, J. La, Mattion, N., 2006. Analysis of the immune response to FMDV structural and non-structural proteins in cattle in Argentina by the combined use of liquid phase and 3ABC-ELISA tests. Vaccine 24, 997–1008. https://doi.org/10.1016/j.vaccine.2005.08.071

Rodríguez, A., Núñez, J.I., Nolasco, G., Ponz, F., Sobrino, F., de Blas, C., 1994. Direct PCR detection of foot-and-mouth disease virus. Journal of Virological Methods 47, 345–349. https://doi.org/10.1016/0166-0934(94)90030-2

Rodriguez, L.L., Gay, C.G., 2011. Development of vaccines toward the global control and eradication of foot-and-mouth disease. Expert Review of Vaccines 10, 377–387. https://doi.org/10.1586/erv.11.4

Roeder, P.L., Le Blanc Smith, P.M., 1987. Detection and typing of foot-and-mouth disease virus by enzyme-linked immunosorbent assay: a sensitive, rapid and reliable technique for primary diagnosis. Research in Veterinary Science 43, 225–232.

Rout, M., Senapati, M.R., Mohapatra, J.K., Dash, B.B., Sanyal, A., Pattnaik, B., 2014. Serosurveillance of foot-and-mouth disease in sheep and goat population of India. Preventive Veterinary Medicine 113, 273–277.

Rowlands, D.J., Sangar, D. V., Brown, F., 1975. A comparative chemical and serological study of the full and empty particles of foot and mouth disease virus. Journal of General Virology 26, 227–238. https://doi.org/10.1099/0022-1317-26-3-227

Rufael, T., Catley, A., Bogale, A., Sahle, M., Shiferaw, Y., 2008. Foot and mouth disease in the Borana pastoral system, southern Ethiopia and implications for livelihoods and international trade. Tropical Animal Health and Production 40, 29–38. https://doi.org/10.1007/s11250-007-9049-6

Sáiz, M., Núñez, J.I., Jimenez-Clavero, M.A., Baranowski, E., Sobrino, F., 2002. Foot-and-mouth disease virus: biology and prospects for disease control. Microbes and infection Institut Pasteur 4, 1183–1192.

Salt, J.S., 1993. The carrier state in FMD an immunological review. Bristish Veterinary Journal 149, 207–223.

Salt, J.S., Mulcahy, G., Kitching, R.P., 1996. Isotype-specific antibody responses to foot-and-mouth disease virus in sera and secretions of “carrier’’ and ‘non-carrier’’ cattle.’” Epidemiology and Infection 117, 349–360.

Sammin, D.J., Paton, D.J., Parida, S., Ferris, N.P., Hutchings, G.H., Reid, S.M., Shaw, A.E., Holmes, C., Gibson, D., Corteyn, M., Knowles, N.J., Valarcher, J.-F., Hamblin, P.A., Fleming, L., Gwaze, G., Sumption, K.J., 2007. Evaluation of laboratory tests for SAT serotypes of foot-and-mouth disease virus with specimens collected from convalescent cattle in Zimbabwe. The Veterinary Record 160, 647–654. https://doi.org/10.1136/vr.160.19.647

Samuel, A.R., Knowles, N.J., 2001. Foot-and-mouth disease type O viruses exhibit genitically and geographically distinct evolutionary lineages (topotypes). Journal of General Virology 82, 609–621.

Samuel, A.R., Knowles, N.J., Mackay, D.K.J., 1999. Genetic analysis of type O viruses responsible for epidemics of foot-and-mouth disease in north africa. Epidemiology and Infection 122, 529–538. https://doi.org/10.1017/S0950268899002265

Samuel, A.R., Ouldridge, E.J., Arrowsmith, A.E.M., Kitching, R.P., Knowles, N.J., 1990. Antigenic analysis of serotype O foot-and-mouth disease virus isolates from the Middle East, 1981 to 1988. Vaccine 8, 390–396.

Sangula, A.K., Siegismund, H.R., Belsham, G.J., Balinda, S.N., Masembe, C., Muwanika, V.B., 2011. Low diversity of foot-and-mouth disease serotype C virus in Kenya: Evidence for probable vaccine strain re-introductions in the field. Epidemiology and Infection 139, 189–196. https://doi.org/10.1017/S0950268810000580

Sanson, R.L., 2005. A survey to investigate movements off sheep and cattle farms in New Zealand, with reference to the potential transmission of foot-and-mouth disease. New Zealand Veterinary Journal 53, 223–233.

Schaible, U.E., Collins, H.L., Kaufmann, S.H.E., 1998. Confrontation between intracellular

bacteria and the immune system. Advances in Immunology 71, 267–377. https://doi.org/10.1016/s0065-2776(08)60405-8

Scott, K.A., Rathogwa, N.M., Capozzo, A. V., Maree, F.F., 2017. Evaluation of immune responses of stabilised SAT2 antigens of foot-and-mouth disease in cattle. Vaccine 35, 5426–5433. https://doi.org/10.1016/j.vaccine.2017.02.003

Scott S, D.C., 2005. Return of the black death: the world's greatest serial killer, 2nd ed. John Wiley & Sons Ltd., West Sussex, England.

Shankar, H., Sharma, S.K., Singh, S. V, Singh, N., Gupta, V.K., 1998. Foot and mouth disease in small ruminants: some epidemiological observations. Indian Journal of Virology 14, 21–25.

Shao, J.J., Wang, J.F., Chang, H.Y., Liu, J.X., 2011. Immune potential of a novel multiple-epitope vaccine to FMDV type Asia 1 in Guinea pigs and sheep. Virologica Sinica 26, 190–197. https://doi.org/10.1007/s12250-011-3174-0

Sharma, G.K., Mohapatra, J.K., Pandey, L.K., Mahajan, S., Mathapati, B.S., Sanyal, A., Pattnaik, B., 2012. Immunodiagnosis of foot-and-mouth disease using mutated recombinant 3ABC polyprotein in a competitive ELISA. Journal of Virological Methods 185, 52–60. https://doi.org/10.1016/j.jviromet.2012.05.029

Shaw, A.E., Reid, S.M., King, D.P., Hutchings, G.H., Ferris, N.P., 2004. Enhanced laboratory diagnosis of foot and mouth disease by real-time polymerase chain reaction. Revue scientifique et technique International Office of Epizootics 23, 1003–1009.

Shirley, M.D.F., Rushton, S.P., 2005. Where diseases and networks collide: Lessons to be learnt from a study of the 2001 foot-and-mouth disease epidemic. Epidemiology and Infection 133,

1023–1032. https://doi.org/10.1017/S095026880500453X

Shukla, R.R., Vaidya, T.N., Joshi, D.D., Pyakural, S., Upadhyaya, K.N., Shukla, R., Valdya, T., Joshi, D., 1974. Studies on the diseases of domestic animals and birds in Nepal. VI. Observations on the epidemiology of an outbreak of foot and mouth diseases at Government Livestock Farm, Khumaltar. Bulletin of Veterinary Science and Animal Husbandry, Nepal 3, 10–15.

Simela, L., Merkel, R., 2008. The contribution of chevon from Africa to global meat production. Meat Science 80, 101–109. https://doi.org/10.1016/j.meatsci.2008.05.037

Sirdar, M.M., Fosgate, G.T., Blignaut, B., Gummow, B., Shileyi, B., Lazarus, D.D., Mutowembwa, P., van der Merwe, D., Heath, L., 2019. A novel method for performing antigenic vaccine matching for foot-and-mouth disease in absence of the homologous virus. Vaccine 37, 5025–5034. https://doi.org/10.1016/j.vaccine.2019.07.002

Smitsaart, E.N., Zanelli, M., Rivera, I., Fondevila, N., Compaired, D., Maradei, E., Bianchi, T., O'Donnell, V., Schudel, A.A., 1998. Assessment using ELISA of the herd immunity levels induced in cattle by foot-and-mouth disease oil vaccines. Preventive Veterinary Medicine 33, 283–296.

Sobrino, F., Sáiz, M., Jiménez-Clavero, M.A., Núñez, J.I., Rosas, M.F., Baranowski, E., Ley, V., 2001. Foot-and-mouth disease virus: A long known virus, but a current threat. Veterinary Research 32, 1–30.

Sommereyns, C., Paul, S., Staeheli, P., Michiels, T., 2008. IFN-lambda (IFN-λ) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. PLoS Pathogens 4. https://doi.org/10.1371/journal.ppat.1000017

Sorensen, K.J., Madsen, K.G., Madsen, E.S., Salt, J.S., Nqindi, J., Mackay, D.K.J., 1998. Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. Archives of Virology 143, 1461–1476.

Srisombundit, V., Tungthumniyom, N., Linchongsubongkoch, W., Lekcharoensuk, C., Sariya, L., Ramasoota, P., Lekcharoensuk, P., 2013. Development of an inactivated 3Cpro-3ABC (mu3ABC) ELISA to differentiate cattle infected with foot and mouth disease virus from vaccinated cattle. Journal of Virological Methods 188, 161–167. https://doi.org/10.1016/j.jviromet.2012.12.016

Stegeman, A., Bouma, A., Elbers, A.R.W., de Jong, M.C.M., Nodelijk, G., de Klerk, F., Koch, G., van Boven, M., 2004. Avian Influenza A Virus (H7N7) Epidemic in The Netherlands in 2003: Course of the Epidemic and Effectiveness of Control Measures. The Journal of Infectious Diseases 190, 2088–2095. https://doi.org/10.1086/425583

Stenfeldt, C., Segundo, F.D.S., de los Santos, T., Rodriguez, L.L., Arzt, J., 2016. The pathogenesis of foot-and-mouth disease in pigs. Frontiers in Veterinary Science 3, 41. https://doi.org/10.3389/fvets.2016.00041

Stenger, S., 2001. Cytolytic T cells in the immune response to Mycobacterium tuberculosis. Scandinavian Journal of Infectious Diseases 33, 483–487. https://doi.org/10.1080/00365540110026584

Sutmoller, P., Thomson, G.R., Hargreaves, S.K., Foggin, C.M., Anderson, E.C., 2000. The foot-and-mouth disease risk posed by African buffalo within wildlife conservancies to the cattle industry of Zimbabwe. Preventive Veterinary Medicine 44, 43–60.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution. https://doi.org/10.1093/molbev/msm092

Tekleghiorghis, T., Moormann, R.J.M., Weerdmeester, K., Dekker, A., 2016. Foot-and-mouth Disease Transmission in Africa: Implications for Control, a Review. Transboundary and Emerging Diseases 63, 136–151. https://doi.org/10.1111/tbed.12248

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Thomson, G., 1994. Foot-and-mouth disease, in: Infectious Diseases of Livestock, Oxford University Press, South Africa. pp. 825–952.

Thomson, G.R., 2009. Currently important animal disease management issues in sub-Saharan Africa. The Onderstepoort Journal of Veterinary Research 76, 129–134.

Thomson, G.R., 2008. A short overview of regional positions on foot-and-mouth disease control in southern Africa., Transboundary animal disease and market access: future options for the beef industry in southern Africa. Institute of Development Studies, UK.

Thomson, G.R., Penrith, M.L., Atkinson, M.W., Atkinson, S.J., Cassidy, D., Osofsky, S.A., 2013. Balancing Livestock Production and Wildlife Conservation in and around Southern Africa's Transfrontier Conservation Areas. Transboundary and Emerging Diseases 60, 492–506. https://doi.org/10.1111/tbed.12175

Thomson, G.R., Vosloo, W., Bastos, A.D.S.S., 2003. Foot and mouth disease in wildlife. Virus Research 91, 145–161. https://doi.org/10.1016/S0168-1702(02)00263-0

Thomson GR and Bastos ADS, 2004. Foot and Mouth Disease, in: Infectious Diseases of Livestock, 2nd Edn Oxford University Press, South Africa. pp. 1324–1365.

Thrusfied M, 2005. Domestic testing, in: Veterinary Epidemiology. Oxford, UK.

Tizard I R, 2013. Veterinary Immunology. Elsevier Health Sciences, St Louis.

Tosh, C., Hemadri, D., Sanyal, A., Bandyopadhyay, S.K., 2003. Genetic and antigenic analysis of two recently circulating genotypes of type A foot-and-mouth disease virus in India: Evidence for positive selection in the capsid-coding genes. Archives of Virology 148, 853–869.

Tsaglas, E., 1995. The recent FMD epizootic in Greece, in: Report of the 31st Session of the European Commission for the Control of Foot-and-Mouth Disease, 5-7 April 1995, FAO, Rome, Appendix 2, 35-40. pp. 35–40.

Uddowla, S., Pacheco, J.M., Larson, C., Bishop, E., Rodriguez, L.L., Rai, D.K., Arzt, J., Rieder, E., 2013. Characterization of a chimeric foot-and-mouth disease virus bearing a bovine rhinitis B virus leader proteinase. Virology 447, 172–180. https://doi.org/ 10.1016/j.virol.2013.08.035

Van Kerkhove, M.D., Vong, S., Guitian, J., Holl, D., Mangtani, P., San, S., Ghani, A.C., 2009. Poultry movement networks in Cambodia: Implications for surveillance and control of highly pathogenic avian influenza (HPAI/H5N1). Vaccine 27, 6345–6352.

Van Vlijmen, H.W.T., Curry, S., Schaefer, M., Karplus, M., 1998. Titration calculations of foot-and-mouth disease virus capsids and their stabilities as a function of pH. Journal of Molecular

Biology 275, 295–308. https://doi.org/10.1006/jmbi.1997.1418

Vangrysperre, W., De Clercq, K., 1996. Rapid and sensitive polymerase chain reaction based detection and typing of foot-and-mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viru. Archives of Virology 141, 331–344. https://doi.org/ 10.1007/BF01718403

Veterinary, C., Van Maanen, C., Terpstra, C., 1989. Comparison of a liquid-phase blocking sandwich ELISA and a serum neutralization test to evaluate immunity in potency tests of foot-and-mouth disease vaccines. Journal of Immunological Methods 124, 111–119. https://doi.org/10.1016/0022-1759(89)90192-0

Vosloo, W., Bastos, A.D., Kirkbride, E., Esterhuysen, J.J., Van Rensburg, D.J., Bengis, R.G., Keet, D.W., Thomson, G.R., Janse Van Rensburg, D., Bengis, R.G., Keet, D.W., Thomson, G.R., 1996. Persistent infection of African buffalo (Syncerus caffer) with SAT-type foot-and-mouth disease viruses: rate of fixation of mutations, antigenic change and interspecies transmission. The Journal of General Virology 77 (Pt 7), 1457–1467.

Vosloo, W, Bastos, A.D.S., Sangare, O., Hargreaves, S.K., Thomson, G.R., 2002. Review of the status and control of foot and mouth disease in sub-Saharan Africa. Revue Scientifique et Technique International Office of Epizootics 21, 437–449.

Vosloo, Wilna, Boshoff, K., Dwarka, R., Bastos, A., 2002. The possible role that buffalo played in the recent outbreaks of foot-and-mouth disease in South Africa. Annals Of The New York Academy Of Sciences 969, 187–190.

Vosloo, W., Hong, N.T.T., Fosgate, G.T., Jacqueline, M.M., Jianning, W., Van Phuc, K., Ngon,

Q.V., Phuong, L.T.T., Hung, D., Hanh, T.X., Van Hung, V., Anh, L.T.Q., Tien, M.T.M., Quang, L.T.V., Long, N.T., Nagendrakumar, S.B., 2015. Efficacy of a high potency O1 Manisa monovalent vaccine against heterologous challenge with a FMDV O Mya98 lineage virus in pigs 4 and 7 days post vaccination. Vaccine 33, 2778–2785. https://doi.org/10.1016/j.vaccine.2015.04.045

Vosloo, W., Thompson, P.N., Botha, B., Bengis, R.G., Thomson, G.R., 2009. Longitudinal study to investigate the role of impala (Aepyceros melampus) in foot-and-mouth disease maintenance in the Kruger National Park, South Africa. Transboundary and Emerging Diseases 56, 18–30.

Vosloo, W., Thomson, G.R., 2017. Natural Habitats in which Foot-and-mouth Disease Viruses are Maintained, in: Foot and Mouth Disease Virus: Current Research and Emerging Trends. pp. 179–210. https://doi.org/10.21775/9781910190517.09

Wang, C.Y., Chang, T.Y., Walfield, A.M., Ye, J., Shen, M., Chen, S.P., Li, M.C., Lin, Y.L., Jong, M.H., Yang, P.C., Chyr, N., Kramer, E., Brown, F., 2002. Effective synthetic peptide vaccine for foot-and-mouth disease in swine. Vaccine 20, 2603–2610. https://doi.org/10.1016/S0264-410X(02)00148-2

Ward, G., Rieder, E., Mason, P.W., 1997. Plasmid DNA encoding replicating foot-and-mouth disease virus genomes induces antiviral immune responses in swine. Journal of Virology 71, 7442–7447. https://doi.org/10.1128/jvi.71.10.7442-7447.1997

Waters, R.A., Fowler, V.L., Armson, B., Nelson, N., Gloster, J., Paton, D.J., King, D.P., 2014. Preliminary validation of direct detection of foot-and-mouth disease virus within clinical samples using reverse transcription Loop-mediated isothermal amplification coupled with a

simple lateral flow device for detection. PLoS ONE 9. https://doi.org/10.1371/journal.pone.0105630

Webb, C.R., 2006. Investigating the potential spread of infectious diseases of sheep via agricultural shows in Great Britain. Epidemiology and Infection 134, 31–40. https://doi.org/10.1017/S095026880500467X

Webb, C.R., 2005. Farm animal networks: Unraveling the contact structure of the British sheep population. Preventive Veterinary Medicine 68, 3–17.

Windsor, M.A., Carr, B.V., Bankowski, B., Gibson, D., Reid, E., Hamblin, P., Gubbins, S., Juleff, N., Charleston, B., 2011. Cattle remain immunocompetent during the acute phase of foot-and-mouth disease virus infection. Veterinary Research 42, 108. https://doi.org/10.1186/1297-9716-42-108

Wolf, T.E., Lazarus, D.D., Opperman, P., Heath, L., Ganswindt, A., Fosgate, G.T., 2020. Impact of foot-and-mouth-disease on goat behaviour after experimental infection with serotype SAT1 virus. Preventive Veterinary Medicine 176, 104912. https://doi.org/10.1016/j.prevetmed.2020.104912

Yamazaki, W., Mioulet, V., Murray, L., Madi, M., Haga, T., Misawa, N., Horii, Y., King, D.P., 2013. Development and evaluation of multiplex RT-LAMP assays for rapid and sensitive detection of foot-and-mouth disease virus. Journal of Virological Methods 192, 18–24.

Yang, M., Caterer, N.R., Xu, W., Goolia, M., 2015. Development of a multiplex lateral flow strip test for foot-and-mouth disease virus detection using monoclonal antibodies. Journal of Virological Methods 221, 119–126. https://doi.org/10.1016/j.jviromet.2015.05.001

Yang, M., Goolia, M., Xu, W., Bittner, H., Clavijo, A., 2013. Development of a quick and simple detection methodology for foot-and-mouth disease virus serotypes O, A and Asia 1 using a generic RapidAssay Device. Virology Journal 10, 125. https://doi.org/10.1186/1743-422X-10-125

Zaikin D G, 1959. Clinical picture of foot-and-mouth disease in sheep under pasture maintenance conditions. Veterinariya 36, 32–34.

Zhang, Z., Pan, L., Ding, Y., Zhou, P., Lv, J., Chen, H., Fang, Y., Liu, X., Chang, H., Zhang, J., Shao, J., Lin, T., Zhao, F., Zhang, Y., Wang, Y., 2015. Efficacy of synthetic peptide candidate vaccines against serotype-A foot-and-mouth disease virus in cattle. Applied Microbiology and Biotechnology 99, 1389–1398. https://doi.org/10.1007/s00253-014-6129-1

APPENDIX A: AEC APPROVAL

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Epidemiology, vaccination and control of foot-and-mouth disease (FMD) in goats in the Greater KNP Area, South Africa**
PROJECT NUMBER	**V022-17 (Amendment 2)**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. DD Lazarus**

STUDENT NUMBER (where applicable)	**U_10675702**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES/SAMPLES	**Goats**
NUMBER OF ANIMALS	**40**
Approval period to use animals for research/testing purposes	**September 2018 –September 2019**
SUPERVISOR	**Prof. G Fosgate**

KINDLY NOTE:

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

APPROVED	Date 8 October 2018
CHAIRMAN: UP Animal Ethics Committee	Signature Nicky Bennett

S*4285-15*

APPENDIX B: HUMANITIES ETHICS APPROVAL

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Office of the Deputy Dean

4 July 2017

Dear Mr Lazarus

Project:	**Epidemiology, vaccination and control of foot-and-mouth (FMD) in goats at the Greater KNP Area, South Africa**
Researcher:	**DD Lazarus**
Supervisors:	**Prof GT Fosgate and Dr PA Opperman**
Department:	**Production Animal Studies (Veterinary Science)**
Reference number:	**10675702 (GW20170623HS)**

Thank you for the **well written** application that was submitted for ethics review.

I am pleased to inform you that the above application was **approved** by the **Research Ethics Committee** on 29 June 2017.Data collection may therefore commence.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should the actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely

Prof Karen Harris
Acting Chair: Research Ethics Committee
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za

cc: Prof GT Fosgate (Promoter)
Dr PA Opperman (Co-Promoter)

APPENDIX C: QUESTIONNAIRE

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Veterinary Science

Stock card No..... *Date.....*

QUESTIONNAIRE FOR SMALL RUMINANT MOVEMENT NETWORK WITHIN THE MNISI COMMUNITY SOUTH AFRICA

The University of Pretoria, Faculty of Veterinary Science is conducting a study on the movement network and trade patterns of small ruminants within the Mnisi (Bushbuckridge) study region, Mpumalanga, South Africa.

You have been selected as one of our respondents to kindly answer the questions with your consent and personal experience. The answers provided will be kept strictly confidential and will be used for research and planning purposes.

Kindly tick √ □ to indicate that the respondent consent to participate in this study.

Thank you for your cooperation.

SECTION A (OWNER DEMOGRAPHIC)

1. Name (optional).....
2. Location.....
3. Dip tank GPS coordinates……...
4. Residence GPS coordinates…….
5. Gender □ M □ F
6. Age of respondent…….
7. Marital status □ Single □ Married □ Divorced □ Widow
8. The highest level of education that you have completed……..

9. Main occupation

   □ Livestock □ Crops □ Government employee □ Private sector □ Own business □ General employee □ House keeper □ Student □ No response Other (specify).....

SECTION B (HERD DEMOGRAPHIC)

1. How many of these animals do you have today?

Species	Cattle	Sheep	Goat	Pig	Chicken	Dog	Cat
Numbers							

2. When did you start rearing animals……? Year……..?
3. What is your motivation for rearing animals…….....
   …….?

SECTION C (ANIMAL MANAGEMENT)

4. Do you graze animals collectively?

   □ Yes □ No □ Unsure
5. If yes, then which animals……?
6. Do you provide your animals with supplemental feed?

   □ Yes □ No □ Unsure
7. If yes, what is your source of supplemental feed…….?
8. When your sheep/goats are sick what do you do?

   □ Call a vet. □Treat yourself □ Sell □ Allow to recover □ Slaughter
9. When your pigs are sick what do you do?

   □ Call a vet. □Treat yourself □ Sell □ Allow to recover □ Slaughter

SECTION D (ANIMAL MOVEMENTS)

10. Which of these animals have you bought in the past 12 months?

Herd Entries: during previous 12 months

Movement	Species	Number	Source farm (Location)	Reason

1 = herd improvement, 2 = gift, 3 = inheritance, 4 = adoption

11. When was the most recent time you introduced a new sheep/goat into your flock …….?

□ None

12. When was the most recent time you introduced a new cattle to your herd……?

□ None

13. When you buy new animals, do you keep them separate for a time?

□ Yes □ No □ Unsure

14. Which of these animals have you sold from your herd in the previous 12 months?

Herd Exits: during previous 12 months

Movement	Species	Number	Destination (Location)	Reason

1 = pay Lobola, 2 = pay school fees, 3 = pay medical bills, 4 = consumption

15. When was the most recent time you sold sheep/goat from your herd……?

□ None

16. When was the most recent time you sold cattle from your herd…….?

□ None

17. Are you required to obtain a movement permit before sheep/goats leave your farm?

□ Yes □ No

18. Are you required to obtain a movement permit when moving pigs from your farm?

□ Yes □ No

19. Are you required to obtain a movement permit when moving chickens from your farm?

□ Yes □ No

20. Do you sale sheep/goats out of your flock when there is outbreak in the community?

□ Yes □ No

21. Who do you usually sale your animals to?

□ Butcher □ Middle man □ Auction

SECTION E (ANIMAL LOSSES)

Herd Losses during previous 12 months

Lose	Species	Number	Reason

1= illness/death, 2 = consumption, 3 = theft, 4 = predation,