Foot-and-Mouth Disease (FMD) vaccination and control in cattle at the wildlife/livestock interface of the Mnisi communal area

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C. Abbreviations

BEI	Binary ethyleneimine
BHK	Baby hamster kidney cell line
BTY	Bovine thyroid primary cell line
C-ELISA	Competitive ELISA
CI	Confidence Interval
CsCl	Caesium chloride
DAFF	Department of Agriculture, Forestry and Fisheries
ELISA	Enzyme linked-immunosorbent assay
FAO-ECTAD	Food and Agriculture Organisation Emergency Centre for Transboundary Animal Diseases
FMD	Foot and mouth disease
FMDV	Foot and mouth disease virus
GLTFCA	Great Limpopo Transfrontier Conservation Area
GLTP	Great Limpopo Transfrontier Park
IB-RS-2	Pig kidney cell line
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQR	Interquartile range
ISRDS	Integrated Sustainable Rural Development Strategy
KNP	Kruger National Park
K-W	Kruskal-Wallis test
L K	Lamb kidney cell line

LFD	Lateral flow device
LID	Livestock in development
LNP	Limpopo National Park
LPBE	Liquid-phase blocking ELISA
MAb	Monoclonal antibody
MVPK-1	Pig kidney cell line
NSPs	Non-structural proteins
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PD_{50}	Protective dose 50
PEG	Polethylene glycol
pH	Negative log of hydrogen ion concentration
RNA	Ribonucleic acid
RT-LAMP	Reverse transcription loop-mediated amplification
RT-PCR	Reverse transcriptase polymerase chain reaction
SADC	Southern African Development Community
SADC-FMD	Southern African Development Community Foot and mouth disease
SAT	South African Territories
TADP	Transboundary Animal Disease Programme
TADs	Transboundary Animal Diseases
$TCID_{50}$	Tissue culture infective dose 50
TMB	Tetramethylbenzidine
VNT	Virus neutralisation test
VP	Variable protein
WAHID	World Animal Health Information Database

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Abstract

Foot-and-mouth Diseases (FMD) is an important livestock disease with economic implications on trade. In southern Africa, the epidemiology of FMD is complicated as a result of the role of African buffalo (*Syncerus caffer*) in the maintenance and transmission of the South African Territories (SAT) virus serotypes. The aim of this study was to evaluate the FMD vaccination of cattle at the wildlife/livestock interface.

A structured questionnaire was administered to communal farmers through in-person interview using the local language (Shangaan) to evaluate their perceptions concerning the current FMD vaccination programme. Cross-sectional sampling by cluster at herd levels was used to estimate proportions of cattle with high titres to FMDV-structural proteins, which was assumed to indicate an immunological response to vaccine routinely administered bi-annually

in the absence of recently recorded outbreaks. A prospective cohort study was employed to evaluate immune responses and the duration of antibody responses to an inactivated aqueous trivalent FMD vaccine (SAT 1, SAT 2 & SAT 3) with blood samples collected on fortnightly bases.

One hundred and four farmers responded to the questionnaire with 73% (76/104) being cattle owners while the remainder being hired cattle handlers. The majority of the respondents (79%; 95%CI: 70%-86%) indicated high level of satisfaction with the current animal health programme. The education level of the farmers varied over levels of satisfaction with the median education level being standard 9 (IQR: 2-12) for non-satisfied respondents, standard 3 (IQR: 0-6) for the little satisfied and standard 7 (IQR: 2-11) for the very satisfied respondents (P=0.036). Non-satisfied respondents were more likely to treat sick animals themselves than seek veterinary assistance (P=0.002). The majority of respondents identified the African buffalo as a risk factor for FMD outbreak (92%, 95%CI: 85%-96%). Two hundred and eighty-six cattle were sampled within six months post-vaccination and relative to antibody titre of ≥1.6 Log_{10} (1:40 dilution), 20% (95%CI: 14%-26%) of cattle had serologically converted to SAT 1, 39% (95%CI: 32%-46%) to SAT 2 and 22% (95%CI: 17%-27%) to SAT 3. Overall, only 4%, 15% and 9% of cattle had antibody titre ≥2 log_{10} to SAT 1, SAT 2 and SAT 3 respectively over a median period of 189 days since the most recent vaccination. Within the longitudinal study, few cattle had evidence of pre-existing antibody responses to SAT viruses at the beginning of the study. However, 14 days post-vaccination, the proportion of seropositive cattle (≥2 log_{10} titre) to the three SAT type viruses varied between 39% - 77% with SAT 2 having the highest proportions. Antibody responses peaked up to 98%, 98% and 65% at 42 days post-vaccination for SAT 2, SAT 3 and SAT 1 respectively until starting to decline at 56 days-post-vaccination.

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1 INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Foot-and-mouth disease (FMD) is an economically important disease of livestock and a global threat to national and international trade in livestock and livestock products. FMD is widely distributed in the developing world, in particular in South America, Africa, Asia and the Middle East. The lack of infrastructure, human resources, movement control and vaccines tailored to local conditions render many developing countries particularly vulnerable to the spread and poor control of FMD. The presence of FMD in these countries poses a constant threat to regions of the world such as Western Europe and North America where FMD has been eradicated and large susceptible livestock populations are at risk.

The control of FMD in Southern Africa is complicated by the genetic and antigenic variability of the South African Territories (SAT) FMD viruses and the uncertainty surrounding protection by FMD vaccines (Knowles and Samuel, 2003; Vosloo, et al., 2004). The current vaccination strategies alone have not been effective in preventing outbreaks and therefore southern African countries have invested in the restriction of animal movements (mainly through fencing), where livestock are separated from wildlife by earlier established veterinary cordon fences. Wildlife species are typically confined to reserves and frequent inspection of livestock for FMD is performed in controlled areas adjoining reserves. Improved livestock vaccination programmes, intensive disease surveillance and movement controls are critical to enable these countries to manage FMD and potentially gain access to regional and international markets for livestock and livestock products. Cattle in FMD vaccination zones, such as those in the areas surrounding the Kruger National Park (KNP, South Africa), Limpopo National Park (LNP, Mozambique) and adjacent game farms, are

vaccinated at least twice annually with a chemically inactivated trivalent FMD vaccine (SAT 1, 2 & 3) (Thomson and Bastos, 2004).

Two fundamental aspects of FMD control with vaccination are: 1) the degree of cross protection provided by the vaccine against currently circulating field viruses, and 2) the duration of immunity conferred by the vaccine. Currently, commercially available vaccines do not seem to provide adequate protection against the various antigenic variants of FMD field viruses within the study area. This is substantiated by in-vitro cross-neutralisation studies where sera raised against vaccine strains do not always neutralise field viruses within the same serotype (SADC Secretariat, Directorate: Food Agriculture and Natural Resources., 2009). Furthermore, FMD vaccines are chemically inactivated and therefore are expected to induce a short-lived immunity like most inactivated vaccines (Hunter, 1998). Therefore, animals require frequent vaccinations with subsequent economic and logistical consequences. In addition to ensuring that the relevant cross-protection is induced by an FMD vaccine, the success of any vaccination campaign is reliant on the antigen/adjuvant formulation, a sufficient cold chain and a strategic vaccination schedule.

1.2 Literature review

1.2.1 The FMD Virus

The aetiological agent of FMD is a small, non-enveloped virus belonging to the genus *Aphthovirus* in the family *Picornaviridae* called Foot-and-mouth Disease Virus (FMDV). The virus has a positive-sense single stranded RNA genome of approximately 8400 nucleotides in length, which contains one large open reading frame encoding a polyprotein which is processed to L ^{pro}, the capsid structural variable protein (VP) 1 (1D), VP 2 (1B), VP 3 (1C), and VP 4 (1A) as well as the non-structural proteins: 2A, 2B, 2C, 3A, 3B, 3C ^{pro} and 3D ^{pro} (Belsham, 2005). A high rate of mutation occurs because the FMDV RNA-dependent

RNA polymerase (3D) lacks proof-reading ability (Domingo and Holland, 1997; Drake and Holland, 1999).

Foot-and-mouth Disease Virus naturally infects cloven-hoofed species and camelids (not dromedary camels), and causes an acute illness characterised by fever and lesions in the oral cavity, coronary band, interdigital space and teats in lactating cows (Kitching, 2002b). It is one of the world's 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 response in both vaccinated and non-vaccinated animals. FMDV structural proteins stimulate the production of neutralising antibodies that provide protection against future disease challenges. Antibodies against non-structural proteins do not offer clinical protection (Grubman and Baxt, 2004).

Seven immunologically distinct FMDV serotypes have been described, namely serotypes A, O, C (the so-called European types), Asia -1 and the three South African Territories (SAT) types 1, 2 and 3. Serotypes A, O, C and Asia-1 constitute a distinct lineage separate from the SAT viruses (Vosloo, et al., 2009). This serological classification is based on the inability of the viruses from different serotypes to induce cross protection in animals (Pereira, 1976). However, subsequent research findings have demonstrated antigenic variation within FMDV serotypes (Mateu, et al., 1988; Samuel, et al., 1990; Samuel and Knowles, 2001; Tosh, et al., 2003).

1.2.2 Characteristics of FMD virus

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 assymetrical protomers (Sáiz, et al., 2002). The virion exists as approximately 70% protein and 30% RNA.

It has a relative molecular mass of 8.5 x 10^6 dalton and a sedimentation constant of 146S[1] (Sobrino, et al., 2001).

Picornaviridae are stable at pH between 3 and 9. The FMDV is distinguished from other members of the *Picornaviridae* by its lability at pH below 7 and relative density in CsCl (1.41 – 1.45 g/ml) (Mason, et al., 2003). The virus is labile in mildly acidic solutions and at pH 6.5 the rate of inactivation has been observed to be 90% per hour while at pH 6.0 or pH 5.0, 90% within a minute (Bachrach, et al., 1957).

In contrast to the effect of pH, FMDV is relatively resistant to the effect of heat with considerable variation among virus types and strains (Thomson and Bastos, 2004). Generally, temperature above 43°C causes rapid destruction of the virus in aerosol, while it survives well in aerosol at cool environmental temperatures with relative humidity above 60% (Sobrino, et al., 2001; Thomson and Bastos, 2004).

1.3 The epidemiology of FMD

1.3.1 Worldwide distribution of FMDV

Foot-and-mouth disease serotypes are not uniformly distributed in the regions of the world where the disease still occurs. In Africa, six of the seven FMDV serotypes occurred and the reported distribution of the outbreaks by country and types since 1948 has been reviewed elsewhere (Vosloo, et al., 2002). There is no doubt, however, that FMD is underreported in the continent and therefore the currently available information is incomplete. Nevertheless, it is clear that serotypes O, SAT 1 and SAT 2 are widely distributed while serotypes A and SAT 3 have a more restricted distribution. Serotype C currently does not appear to be in circulation. Disease in livestock caused by SAT 3 has not been reported outside southern Africa despite having been reported in buffalo in East Africa in the 1970s (Hedger, et al.,

[1] This is defined as measured antigen concentration of each batch of vaccine by the sedimentation gradient procedure.

1973). Of the three SAT types, SAT 2 has been reported most frequently in domestic animals: between 1900 and 1987, it was the cause of 48% of outbreaks in livestock in southern Africa that were typed. SAT 1 and SAT 3 accounted for 36% and 16% respectively, (Thomson, 1994).

As stated previously, six of the seven FMDV serotypes (O, A, C, SAT 1, SAT 2 & SAT 3) have occurred in Africa, but type C has not been isolated in the world since 2004. So only 5 serotypes are recovered currently in Africa, while Asia has mainly outbreaks caused by 3 serotypes (O, A & Asia-1), and South America with only 2 (O, A) (Rweyemamu, et al., 2008). The continents Europe, North-America, Australia and Antarctica are currently free of FMDV infection. There are periodic incursions of serotypes SAT 1 and SAT 2 from Africa into the Middle East (Donaldson, 1999; Valarcher, et al., 2004).

1.3.2 FMD situation in Southern Africa

Livestock farming is important to the rural economy of most Southern African Development Community (SADC)[2] member states. More than 75% of livestock production in this region is under non stationary management and is therefore prone to numerous challenges including animal diseases that reduce livestock productivity (SADC Foot and Mouth Disease and FAO-ECTAD, 2008). Transboundary animal diseases[3] (TADs), including FMD, have greater impacts on intensive farming systems. In extensive systems, disease due to FMDV infection is often mild and of little concern to animal owners. Three serotypes of FMD, SAT 1, 2 & 3 are maintained within the African buffalo (*Syncerus caffer*) population within the SADC region, with serotypes O and A occurring in cattle in Tanzania. Evidence suggests that SAT viruses evolved in buffalo in sub-Saharan Africa while serotypes A, O, C & Asia-1 evolved

[2] The SADC member states are: Angola, Botswana, Democratic Republic of Congo, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Seychelles, South Africa, Swaziland, United Republic of Tanzania and Zimbabwe.

[3] These are animal diseases that are of significant economic, trade and/or food security importance, which can easily spread beyond national borders and have potential to reach epidemic proportions and their control and management including exclusion, requires coordinated efforts in more than one country.

in livestock (Vosloo, et al., 1996; Bastos, et al., 2001; Bastos, et al., 2003). It is for this reason that most SADC member states are considered endemically infected, limiting prospects for international and regional trade in livestock and livestock products.

Since 2000, there has been an increase in the distribution and occurrence of FMD outbreaks necessitating the development of a regional strategy for progressive management of FMD in sub-Saharan Africa (OIE Collaborating Centre for Training in Integrated Livestock and Wildlife Health & Management, 2011). Southern African countries have developed complicated methods to deal with FMD (Thomson and Bastos, 2004). South Africa, like Botswana and Namibia had previously obtained recognition from the World Organisation for Animal Health (OIE) for zones being "free from FMD without vaccination" until the 2011 outbreak in KwaZulu Natal region. However, the country has recently regained its freedom status for zones free without vaccination on 14th February, 2014 (OIE, 2014).

1.3.3 Transmission of FMD virus

Foot-and-mouth disease is usually spread by the movement of infected animals and contact with contaminated materials. Susceptible cattle coming in contact with infected animals are typically infected by the respiratory route but the virus can also enter through an abrasion on the skin. Cattle are highly susceptible to infection by the respiratory route; however, in reality would be better represented by a probability distribution. The median probability of infection given a single inhaled $TCID_{50}$ was estimated to be 0.031 with 95% Bayesian credibility intervals (CI) of 0.018-0.052 for cattle and 0.045 (CI = 0.024-0.080) for sheep (French, et al., 2002). Calves consuming contaminated milk can also become infected, because the milk of infected cow may contain high concentrations of the virus. Among all susceptible livestock species tested, cattle and sheep are the most likely to become infected by low virus doses applied in aerosols generated by other infected animals. But because cattle have a large

respiratory volume compared to small ruminants they are more likely to become infected by the airborne route (Donaldson, 1987).

1.4 Clinical signs of FMD in cattle

The incubation period for FMD in cattle is usually between 2 and 14 days and varies due to the infecting dose, the strain of virus and the susceptibility of the individual host (Kitching, 2002b). Typically, between-farm transmission has been observed to have a longer incubation period, but once the quantity of virus in the environment increases on an infected farm, the incubation period reduces (Kitching, 2002b). The first clinical sign is pyrexia (approximately 40°C) lasting one or two days. Vesicles subsequently develop on the tongue, hard palate, dental pad, lips, gums, muzzle, coronary band and interdigital space. Vesicles may also develop on the teats of lactating cows as a result of stress and strain induced by suckling offspring or milking. Young calves may die before the appearance of vesicles because of the predilection of the virus to invade and destroy muscle cells of the heart. Acutely infected cattle can salivate profusely due to oral lesions and stamp their feet or lie down and be reluctant to stand due to interdigital and coronary band lesions.

1.5 Laboratory diagnosis of FMDV

The laboratory diagnosis of FMDV can be achieved by virus isolation or demonstration of FMD viral antigen or nucleic acid in clinical samples of tissue or fluid. The detection of virus-specific antibody can also be used for diagnosis and antibodies to viral non-structural proteins (NSPs) are good indicators of infection, irrespective of vaccination status. The NSPs are highly conserved proteins and are therefore not serotypes specific.

1.5.1 FMD antigen detection (Virus isolation)

FMDV will grow in a wide range of primary and continuous in-vitro cell cultures. Primary cells are cells processed straight from tissue with no passage and therefore could contain a mixture of cell types, while continuous cells are purified cell lines. The most sensitive cell

culture for the isolation of FMDV is primary bovine thyroid (BTY) cells (House and House, 1989). Continuous cell lines including baby hamster kidney cell (BHK), lamb kidney (LK) and the pig kidney cell lines IB-RS-2 and MVPK-1 are also susceptible to FMDV infection. The foetal goat cell line (ZZ-R 127) is a sensitive, rapid and convenient medium for the isolation of FMDV and has served as a useful alternative to BTY (Brehm, et al., 2009). The sensitivity of virus isolation as a technique for FMDV diagnosis will depend upon the quality and type of cells used as well as the quality of the sample (Conlan, et al., 2008). Virus isolation is considered the "gold standard" method but it may take up to four days for results and this will delay the confirmation of FMDV.

1.5.2 FMD antigen detection (Sandwich ELISA)

The antigen capture sandwich ELISA is a highly sensitive method for virus detection and typing in clinical materials. It is the test of choice for FMD endemic countries (OIE, 2012a) because it can be automated to process large numbers of samples and be completed within a few hours. The test principle 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 materials are allowed to bind to the capturing antibodies. Bound viruses are detected by serotype-specific tracing antibody. The FMD antigen capture ELISA has been developed to differentiate FMDV serotypes (Roeder and Le Blanc Smith, 1987). The FMD antigen capture ELISA replaced the complement fixation test for primary FMD diagnosis and serotype identification (Ferris and Dawson, 1988). In this test, sample quality is important as lesions older than 4 – 5 days have less virus; however, samples unsuitable for virus isolation can be tested by ELISA. The ELISA is well suited to low technology settings because it does not require live virus and employs robust technology.

1.5.3 Molecular techniques for nucleic acid detection

More than 50 nucleic acid hybridisation and PCR technologies have been reported for the diagnosis of FMD (Reid, et al., 2002). Recently, real time PCR methods (Taq Man, molecular beacons, Primer-Probe Energy Transfer System, RT-PCR in the 3D gene) have been developed for FMD diagnosis and are now the mainstay for FMD genetic diagnosis (Callahan, et al., 2002; Reid, et al., 2002; Moonen, et al., 2003; Oem, et al., 2005; Niedbalski and Kesy, 2010). Evaluation of real time PCR methods with conventional diagnostics (Shaw, et al., 2004; Ferris, et al., 2006) concluded that PCR was generally more sensitive and is ideal for samples that contain low concentrations of virus. Another promising development for developing country laboratories is the one-step, reverse transcription loop-mediated amplification (RT-LAMP) assay, which enables FMD virus to be detected within 1 hour using a single tube without a thermal cycling requirement (Dukes, et al., 2006; Chen, et al., 2011; Yamazaki, et al., 2013).

1.5.4 Lateral flow device

A lateral flow device (LFD) employing monoclonal antibodies has been developed for the detection of FMDV (Ferris, et al., 2009). This test is based upon the principles of immuno-chromotograpy, in which soluble antigens (such as infected clinical materials) are allowed to flow through a porous 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 antibody can be labelled with either colloidal gold or selenium. The fluid then flows through a detection zone containing immobilised antibody against the antigen. The sensitivity for detection of FMDV serotype SAT 2 was enhanced from 65% to 90% when the monoclonal antibody (MAb) 1F10 in the devices was substituted with the MAb 2H6. With a specificity of 99.4% and comparable sensitivity of 88.2% for the detection of FMDV serotype SAT 2 antigens, this device is superior to the slower and more complicated antigen capture ELISA. The LFD procedure is also simple, rapid and easy to perform which means

that it has the potential to be used as a pen-side test for diagnosis and serotyping (Ferris, et al., 2009; Ferris, et al., 2010).

1.5.5 FMD antibody detection (Virus neutralisation test)

The virus neutralisation test (VNT) is a serotype-specific serological test for FMDV. VNTs depend on cell cultures and the use of live virus. With each VNT, a virus titration is included, so that the actual virus titre and virus doses for that test can be determined. VNT estimates the ability of antibody to neutralise the biological activity of antigen when mixed in vitro. Viruses may be prevented from infecting cells after specific antibody has combined with and blocked their critical attachment sites. Serum titres are expressed as the $\log_{10}$ reciprocal of the dilution that protects 50% of cell culture cells from lysis due to the virus. Testing should be performed in bio-containment facilities within a laminar air flow cabinet. VNTs are more prone to variability than ELISAs, because cell cultures and live virus are involved. In addition, VNTs are more time consuming and susceptible to contamination (OIE, 2012a).

1.5.6 FMD antibody detection (Liquid-phase blocking ELISA)

The FMD liquid-phase blocking ELISA (LPBE) was developed for the detection of FMDV-specific antibodies as a replacement of the conventional virus neutralisation test (VNT). The LPBE 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 (the “liquid phase”). The principle for the test is that antibody present in the test serum will block the FMD antigen from subsequent detection. Test results are highly correlated with VNT results (Araujo, et al., 1996) and it has been suggested that the LPBE could be used as an in-vitro method to estimate protection to FMDV challenge (Hamblin, et al., 1986a; Hamblin, et al., 1986b; Hamblin, et al., 1987; 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, 2012a) and is the primary test for determining vaccine titres.

1.5.7 FMD antibody detection (Solid-phase ELISA)

A solid-phase FMD competitive ELISA (C-ELISA) has been developed that can be used for all seven serotypes of FMDV (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. The C-ELISA is more rapid than the LPBE and results can be obtained in the same day (4 – 5 hours). It was found to be more robust and 100% sensitive relative to the LBPE results. It has a specificity of >95% which is superior to the LPBE, and was used during the UK FMD outbreak to allow for rapid screening of serum samples for FMD antibodies (Mackay, et al., 2001; Paiba, et al., 2004). Many other ELISAs have been developed and validated, these include commercially available kits e.g. Prionics types, O, A and Asia-1, others are used in FAO monitoring programmes (Brescia test).

1.5.8 FMD antibody detection (Non-structural protein assays)

Diagnostic assays to differentiate antibodies induced by FMDV infection from those induced by vaccination have been developed (De Diego, et al., 1997). Antibody to expressed recombinant FMD non-structural proteins (NSPs) can be measured by ELISA or immunoblotting. Also one of the OIE standard tests is the South American ELISA combined system of an indirect ELISA-3ABC with an enzyme-linked immuno-electrotransfer blot (EITB) assay (Bergmann, et al., 2000), which involves screening of samples by I-ELISA 3ABC, together with confirmation of suspects or positive samples by EITB. The ELISA employs purified recombinant NSP antigens absorbed directly to microplates to trap antibodies present in samples of infected animals (De Diego, et al., 1997; Sørensen, et al., 1998; Mackay, et al., 2001). The response against NSP is not serotype specific and indicates infection with any of the seven serotypes. The detection of antibodies to the NSPs of FMDV

can be used to identify past or present infection. FMDV vaccinated cattle typically do not develop a detectable antibody response against NSP when vaccinated with a purified vaccine (OIE, 2012a).

1.6 Diagnosis of FMD in South Africa

South Africa has a level 3 bio-containment facility that is used for the diagnosis of highly contagious diseases including FMD (Bruckner, et al., 2002). This is performed at the Transboundary Animal Diseases Programme (TADP) of the Onderstepoort Veterinary Institute, an Institute of the Agricultural Research Council of South Africa. The TADP, since inauguration in 1981 has performed research and diagnosis of FMD in southern Africa. The laboratory has a large data base of partial VP1 gene sequences of FMD virus that has contributed to understanding the epidemiology of the SAT type viruses in southern Africa (Bruckner, et al., 2002).

The laboratory has the capacity for antigen capture ELISA, liquid phase blocking ELISA, non-structural protein ELISA, virus isolation using different cell lines and polymerase chain reaction and genome sequence technology. Additionally, the laboratory is involved in vaccine matching tests and disinfectant efficacy studies. Currently, the laboratory is in the process of upgrading its facilities for FMD vaccine production.

1.7 Current FMD vaccine

The currently applied FMD vaccines are produced by infecting baby hamster kidney-21 cells with virulent FMDV followed by chemical inactivation with binary ethyleneimine (BEI) and purification by ultrafiltration (Doel, et al., 2003). Polyethylene glycol (PEG) precipitation and ultrafiltration can also be used for purification. Antigen is then diluted with buffers and blended with either oil or aluminium hydroxide/saponin adjuvant. Oil-emulsion vaccines are widely used for the vaccination of pigs, whereas, aluminium hydroxide/saponin-adjuvanted vaccines can only be used in ruminants. Although modern BEI inactivation procedures make

FMD vaccines safe for use in the field (Doel, 2003), the requirement for field strain tissue culture adaptation and large volumes of live virus production possess significant bio-containment and biosafety challenges.

The FMD vaccine currently used in South Africa is a trivalent inactivated aluminium hydroxide adjuvanted product containing isolates of SAT 1, 2 & 3. The amount of each antigen generally varies from 1 to 10µg, depending on the antigenicity of the strain. Serotypes O and SATs, require more antigen compared with the serotypes A, Asia 1 and C in order to achieve an equivalent potency (Doel, 2003). In a study conducted at the Pirbright Institute (Surrey, UK), the administration of similar antigen payload per ml failed to protect animals from SAT infection, whereas all type A vaccinated animals were protected upon homologous challenge (Oh, et al., 2006). Variation in potency may be attributed to the unequal stability of antigen for different serotypes of FMDV. The 146S particles have been identified as the immunogenic component of a FMD vaccine and any degradation of these particles may reduce the potency (Doel and Chong, 1982).

1.8 FMD vaccination in cattle

The use of vaccine still remains the mainstay for the emergency and prophylactic control of FMD in endemic areas; however, the integrity of the fences around the KNP is likely to be equally important. For successful control it is essential that the vaccine is of good quality and contains the appropriate vaccine strains that will induce effective immunity against the local antigenic variants. Logistics such as vaccine administration, dose of vaccine, routes of administration and an effective cold-chain are important to ensure appropriate immunisation. Based on an assumed basic reproduction number of 4 for FMD, one needs to protect at least 75% of the animals to reach ae effective reproduction ratio below 1. Effective protection can be achieved by vaccinating a large proportion of the population with a good quality and potent vaccine that matches the circulating field viruses; while the remaining will be

protected by herd immunity. At a population level, the loss of effective herd immunity to FMDV occurs through the introduction of naïve animals into the population and additionally, the decline in immune responses over time in vaccinated animals. In the field, post vaccination duration of immunity ranges from 4-6 months after the last vaccine injection in cattle, particularly with aluminium hydroxide/saponin adjuvant vaccines (Woolhouse, et al., 1996). As a consequence, there is a risk of 'immunity gaps' if national vaccination campaigns are not held with sufficient frequency, lack of a good primary immunization (2 inoculations of vaccine 2-8 weeks apart) or with suboptimal vaccine administration.

For efficient control of FMD, vaccination and restriction of the movement of potentially infected animals and animal products are crucial. In endemic settings, prophylactic vaccination of the susceptible population every 4-6 months may be a suitable option following the primary course of two injections 2-8 weeks. Historically, different susceptible species are treated in different ways to control FMD. Cattle are more susceptible to aerosols and should be vaccinated with single or multiple administrations, as per requirement in free or endemic areas (Parida, 2009).

1.9 Immunity induced by FMD virus and vaccine

Immune responses against FMDV include circulating humoral antibody has been shown to correlate with protection against FMDV (Mackowiak, et al., 1962; van Bekkun, 1969; Pay and Hingley, 1987; McCullough, et al., 1992a; McCullough, et al., 1992b). IgM is the first serum-neutralizing antibody that appears at 3-4 days post infection or vaccination, and peaks in concentration approximately 10-14 days after infection (Collen, 1994; Golde, et al., 2008). IgG is detected 4-7 days post infection or post vaccination and becomes the major neutralizing antibody by 2 weeks following immunisation (Sobrino, et al., 2001). In both vaccinated and infected cattle, the IgG_1 titre has been reported to be higher than IgG_2 (Salt, 1993). The major antibody classes found in secretions of the upper respiratory and GI tracts

are initially IgM, followed by IgA and IgG (Salt, 1993). It is well known that parenterally administered inactivated FMD vaccine in cattle elicits very little or no IgA in mucosal secretions (Archetti, et al., 1995). Oropharyngeal replication of virus in an infected animal, however, stimulates IgA production in saliva, nasal and oropharyngeal secretions (Salt, et al., 1996; Parida, et al., 2006). Although FMDV elicits a rapid humoral response in both naturally infected and vaccinated animals (Grubman and Baxt, 2004), it is slightly faster in natural infection. Protection has been correlated with high levels of neutralising antibody (Ahl, et al., 1990; McCullough, et al., 1992a; Oh, et al., 2006; Brehm, et al., 2008) and is reported to be serotype specific (McCullough, et al., 1992a).

1.10 FMD control zones in South Africa

South Africa is divided into 3 FMD control zones. In the demarcation of these zones, the environment, fences, species and numbers of wild and domestic animals, and types of animal husbandry being practised in the region are considered. If a farm, property, game reserve or conservancy or a part thereof in one zone, forms a unit with no fences between them with a farm, property, game reserve or conservancy or a part thereof, specified in another zone, the entire unit will automatically fall within the zone having the highest FMD risk (Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health, 2012).

1.10.1 Infected zone

The infected zone is a clearly defined geographical area within the Republic of South Africa in which FMD is endemic due to the presence of FMD carrier buffaloes. This forms the Kruger National park and other game reserves where only wildlife is found. Buffaloes are confined to the park and their movement is restricted. The 2011 outbreak in the KwaZulu Natal Province required the designation of a new infected zone encompassing the communal area in which FMD was recognised.

1.10.2 Protection zone

In the Republic of South Africa, the protection zone does not have free zone status as per the OIE definitions (OIE, 2012b). It is a clearly defined geographic area between the infected and free zones and it is divided into two sub-zones.

1.10.2.1 Protection zone with vaccination

The protection zone with vaccination is a clearly defined geographical area adjacent to the infected zones (Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health, 2012). This includes areas surrounding parks and game reserves where livestock are reared with cordon fences as the only barrier between wildlife and livestock. Routine FMD vaccination of cattle is performed and no buffaloes are allowed in the zone. Strict movement control of live animals and products is practised in addition to intensive weekly FMD surveillance.

1.10.2.2 Protection zone without vaccination

The protection zone without vaccination is a clearly defined geographical area adjacent to the free zone and some international boundaries. Only FMD free buffaloes are allowed in the zone and movement is subject to specific requirements for fencing and regular testing (Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health, 2012). Strict movement control of live animals and products with frequent FMD surveillance is also applicable in this zone on a fortnightly bases.

1.10.3 Free zone

This is a clearly defined geographical area comprising the entire Republic of South Africa, excluding infected and protection zones.

1.10.3.1 Inspection area of the free zone

This is a clearly defined geographical area within the free zone, adjacent to the protection zone and some international boundaries. It forms part of the controlled area where movement control of live animals and regular FMD surveillance is conducted.

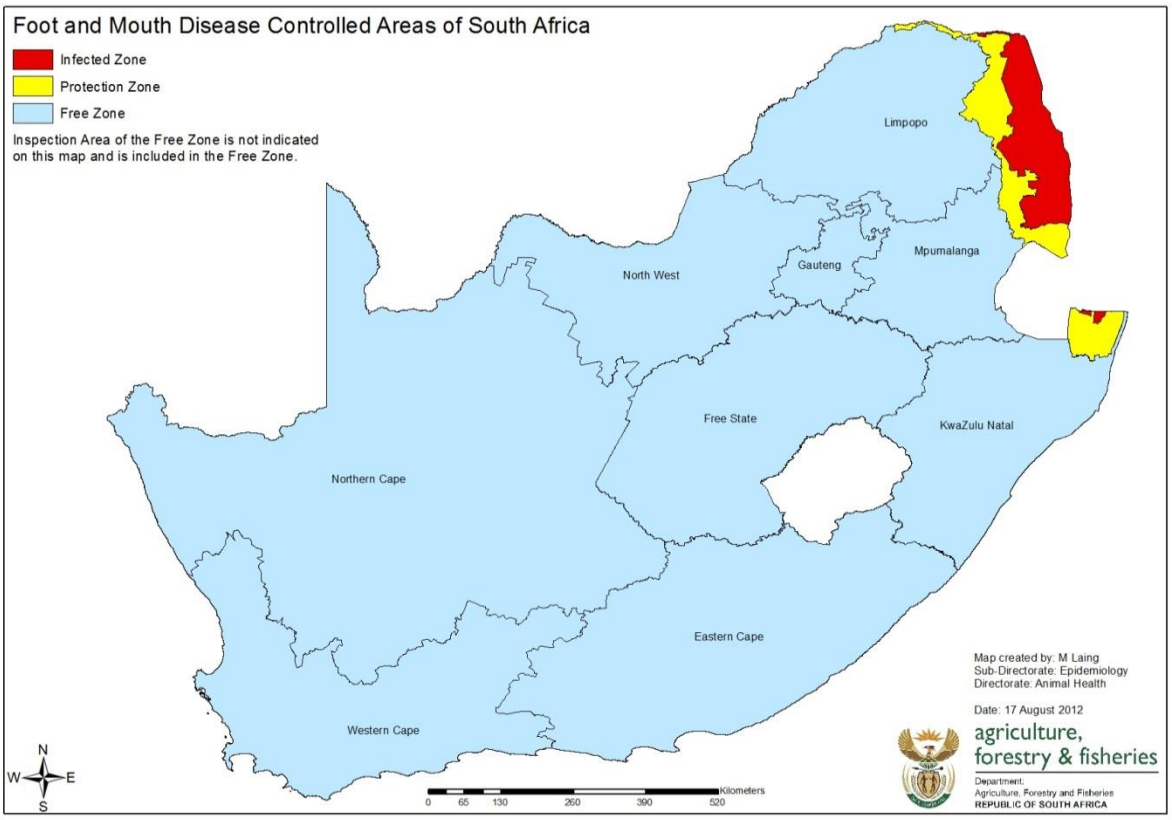

Figure 1. Map of South Africa showing the FMD control zone surrounding the KNP Provided by the DAFF Directorate of Animal Health 2013

1.11 FMD control in South Africa

FMD control measures includes the separation of wildlife from susceptible livestock populations using electrified (or high impact non-electrified) fences, clinical surveillance of susceptible livestock, routine vaccination and movement control of susceptible livestock, wildlife and livestock products.

Control interventions are a combination of one or more of the outlined measures and depend upon the location within the country.

In the protection zone with vaccination areas of the country, cattle are inspected at designated dip tanks every 7 days with small stock (i.e. goats, sheep and pigs) inspected every 28 days.

Upon suspicion of the disease, serological and virological surveillance will be instituted in accordance with the current FMD Contingency Plan (Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health, 2012).

All cattle, excluding calves presumed to have maternal antibodies should be vaccinated every 4 months against FMD according to directions for use of the vaccine, including revaccination of first time vaccinated cattle after 3-4 weeks (Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health, 2012). However, this is not always practiced in the field. 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 previous vaccination in the form of vaccination record. As an identity, a permanent "**F**" is branded on the right side of the neck of each cattle when vaccinated for the first time.

Disease control fences, that prevent contact between infected and susceptible animals are maintained according to regulations and regularly inspected by veterinary officials (Jori, et al., 2011).

1.12 The significance of the study

Recent research findings suggest that the current FMD vaccine used in SADC has poor performance, including a short duration of immunity and possibly inappropriate antigenic variants (Hunter, 1998; SADC Secretariat, Directorate: Food Agriculture and Natural Resources., 2009). The topotypes included in the current FMD vaccine have not been fully known in relation to the field viruses occurring in the study area and the Great Limpopo Transfrontier region. This gap in knowledge prevents agencies from making informed decisions concerning mitigation strategies related to the control of FMD in the region.

1.13 Aims and objectives

The overall objective of this study was to determine the proportion of cattle vaccinated against FMDV within the Mnisi community of the Great Limpopo Transfrontier Park (GLTP) wildlife/livestock interface.

Specific objectives of this study were:

- To determine the herd-level proportion of cattle vaccinated against FMDV from owner-stock card and livestock register and evaluate perceptions and knowledge-base of farmers concerning the current FMD control programme within the Mnisi study area.
- To estimate the association between reported vaccination history and liquid-phase blocking ELISA titres to SAT 1, SAT 2, and SAT 3 viruses.
- To determine the effectiveness of the currently applied vaccination protocol[4] for the induction of effective humoral immune responses based on liquid phase blocking ELISA titres to SAT 1, SAT 2, and SAT 3 viruses.

[4] Three rounds of vaccination are administered at four monthly intervals as a mass campaign within the control areas.

2 PERCEPTIONS OF COMMUNAL FARMERS CONCERNING FOOT-AND-MOUTH DISEASE EPIDEMIOLOGY AND CONTROL

2.1 Introduction

In Africa, the livestock husbandry system practiced in communal areas is typically extensive using indigenous breeds. These breeds are often less productive compared to the intensively reared livestock but they often have greater disease resistance (Scholtz, 1988) and can survive under harsh environmental conditions (Mapiye, et al., 2007). Resource-poor farmers frequently employ communal livestock production systems at interfaces with protected wildlife areas (Osofsky, 2005). Production outputs at interface areas are often low because of husbandry practices, pasture quality and transmission of infectious diseases (Caron, et al., 2013).

Contacts among people, livestock and wildlife occur at interface areas (Brahmbhatt, et al., 2012; de Garine-Wichatitsky, et al., 2012). Interspecies contacts can increase the risk of disease transmission, which is a concern for communal farmers (de Garine-Wichatitsky, et al., 2012). Communal farmers raise livestock to produce milk, meat, hides and manure that can be used to fertilise crop production (Barrett, 1992; Chimonyo, et al, 1999; Dovie, et al., 2006). Cattle also provide draught power for the cultivation of crops and transport of goods and services (Bayer, et al., 2004; Shackleton, et al., 2005). Livestock have been described as “inflation free banking” for resource-poor people and can be sold to pay for school fees, medical bills, village taxes and other household expenses (Dovie, et al., 2006). Livestock farming reduces household food insecurity and poverty in communal areas (ISRDS, 2004; Coetzee, et al., 2006).

Wildlife species are potential reservoirs for livestock diseases and this can be a challenge for livestock production at the interface with protected areas. Some TADs are zoonotic and these diseases at the interface have a negative impact on resource-poor farmers because they reduce

human productivity and can cause mortality (de Garine-Wichatitsky, et al., 2012; Caron, et al., 2013). Diseases that only affect livestock can cause direct mortality or reduced productivity.

Disease control at the livestock wildlife interface often includes vaccination and must consider issues related to vaccine delivery (LID, 1998; Heffernan and Misturelli, 2000) and characteristics of the farmers including perceptions and awareness of the control methods in question (Bhattacharyya, et al., 1997; Bolorunduro, et al., 2004; Fandamu, et al., 2006; Homewood, et al., 2006). Important aspects to the adoption of animal health interventions among the poor are access, affordability and acceptability (Heffernan and Misturelli, 2000). The goal of vaccination campaigns is a wide-scale adoption at the community, national and even global levels (Mason and McGinnis, 1990; Humair, et al., 2002) and therefore must consider the perceptions and feelings of the resource-poor farmers (McLeod and Rushton, 2007; Heffernan, et al., 2008).

The cattle of resource-poor farmers in Africa can be affected by foot-and-mouth disease (FMD) (Jibat, et al., 2013), an important TAD in Africa (Vosloo, et al., 2002a; Thomson and Bastos, 2004), and control is necessary for poverty alleviation and improved food security. The majority of milk and meat consumed at the communal level is produced locally (Perry and Grace, 2009) and while FMD is typically considered an insignificant disease in extensive systems (Ferguson, et al., 2013), there is clear evidence that losses from reduced production and market access can be substantial (Kivaria, 2003; Perry and Grace, 2009).

The objective of the present study was to evaluate the perceptions of farmers concerning the epidemiology and control of FMD at the wildlife/livestock interface within Mpumalanga Province, South Africa.

2.2 Materials and methods

2.2.1 Research ethics

Ethical clearance for the questionnaire study was obtained at the University of Pretoria from the Research Ethics Committee (Project Number 2012-04-04) at the Faculty of Humanities.

2.2.2 Study location and population

This study was conducted at 15 livestock inspection points (dip tanks) of the Mnisi Community, Bushbuckridge Municipal Area Mpumalanga Province, South Africa (Fig. 2). The Mnisi Community has a land area of 30,000 ha and a human population of 40,060 people living within 8,555 households. Domestic livestock include 14,400 heads of cattle owned by 1,300 farmers, 6,190 goats owned by 920 farmers and 330 pigs owned by 36 farmers (Statistics SA, 2001). Local household livelihoods are supplemented by land-based activities including cultivating home gardens, rearing livestock and gathering natural resources (Cousins, 1999; Shackleton, 2000; Dovie, et al., 2002).

The Bushbuckridge area has generally sandy and infertile granitic soils. Rainfall occurs mainly during summer months (October-April) and the total amount varies from 800 mm per annum in the west to 500 mm per annum in the east. The increasing aridity moving eastward is accompanied by increasing variability in the mean annual rainfall and drought is a common occurrence in the district (Shackleton, 2000).

The main agricultural activity in the area is livestock farming with cattle as the most important species. Goats and chickens are locally abundant and there are also a few donkey and pig farmers. Two thirds of the land area forms an interface with the Kruger National Park and provincial and private game reserves. Cattle and wildlife are separated by game-proof fences and the entire study region is situated within the FMD protection zone with vaccination. Three wards from the Mnisi Community were selected for study:

Bushbuckridge-1, Bushbuckridge-2 and Bushbuckridge-3. Each ward has a government animal health technician that supervises activities at five community dip tanks.

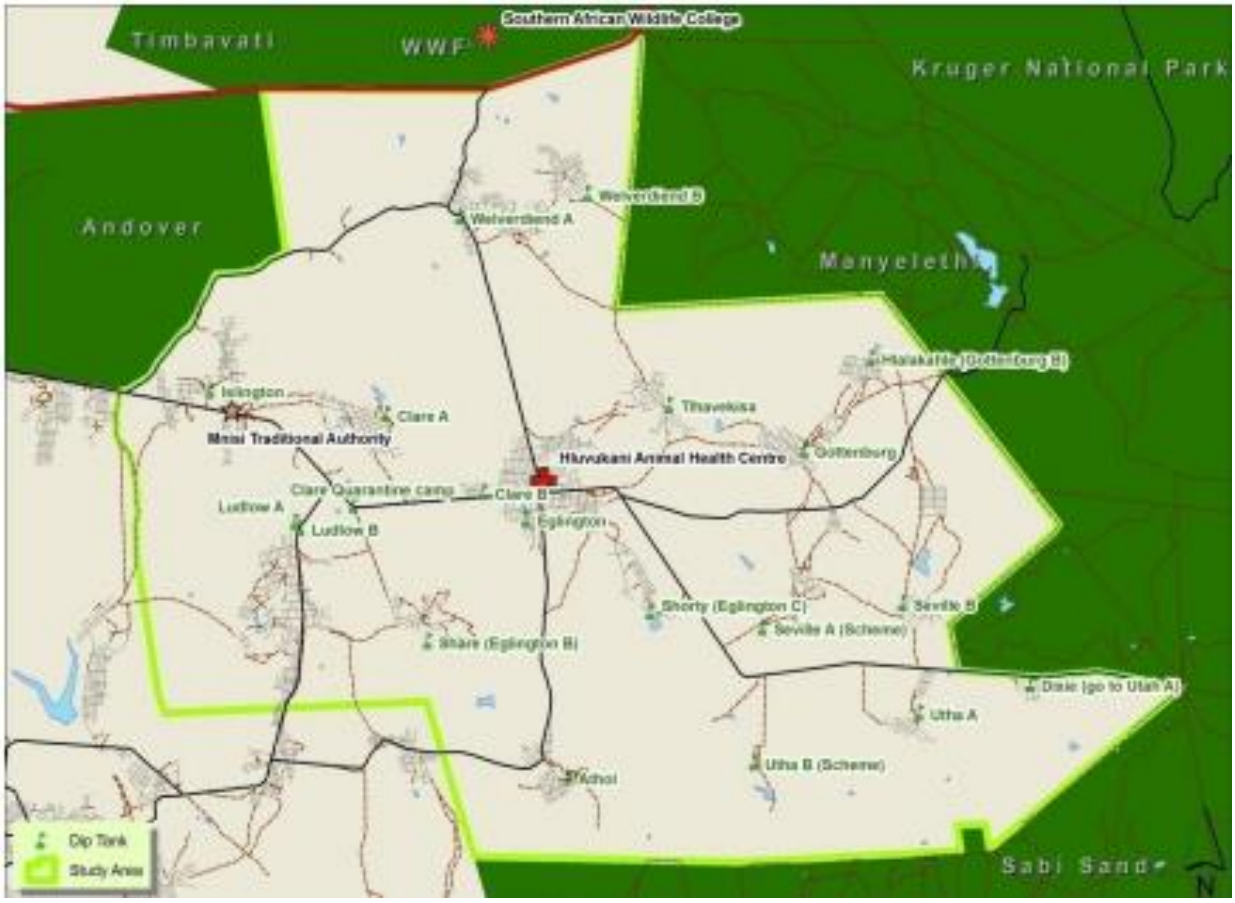

Figure 2. Location of the study area showing the distributions of the community dip tanks Courtesy of the Mnisi Community Programme 2013

2.2.3 Sample size justification

The sample size was calculated to estimate the proportion of respondents with knowledge concerning FMD epidemiology and control. A percentage of 50% was assumed since there was no prior information and was estimated with 10% absolute error at the 95% level of confidence (Open Epi, version 2.3.1, Open Source Epidemiological Statistics for Public Health calculator – SS propor software). The sample size was estimated as 97 but was increased to 104 to allow for the sampling of 10% of total farmers from each dip tank.

2.2.4 Questionnaire development and administration

A semi-structured questionnaire was designed to evaluate perceptions of communal farmers concerning FMD epidemiology and control at the wildlife/livestock interface. The questionnaire included multiple choice, dichotomous (yes/no), ordinal scales and free numerical or text responses focusing on the respondent's level of education and experience. Questions addressed owner demographics, herd management practices, general disease control and knowledge of FMD epidemiology.

Collected socio-demographic data included age, gender, marital status, education level, and sources of household income. Herd management data included the number of livestock, amount of time since the most recent purchase/sale of animals, duration of livestock farming and source of livestock drinking water. General disease control data included knowledge of FMD vaccination, satisfaction with the routine vaccination programme, satisfaction with dipping, favourite dip tank activities and annual frequency of FMD vaccination.

Data were collected concerning knowledge of the clinical signs of FMD, history of previous FMD outbreaks, disease management, and perceived risk factors for FMD outbreaks.

A composite vaccination score was created concerning factors that might affect farmers' participation in a vaccination campaign. This score was a summation with favourable responses assigned +1, unfavourable responses -1 and uncertain responses 0 marks. Questions included: vaccination can reduce disease in cattle, vaccination can make cattle sick, vaccination can cause abortion in cattle, vaccination improves cattle wellbeing, vaccination can reduce feed intake in cattle, sick cattle should be presented for vaccination and pregnant cattle should be presented for vaccination.

Questionnaires were administered through an in-person interview in the local language (Shangaan) after translation from English. Within each community dip tank, 10% of the

registered livestock owners/handlers were conveniently selected as they presented their cattle for inspection. The study was conducted in May and June 2012, a period that coincided with the routine FMD mass vaccination campaign in the area.

Farmers were eligible for enrolment if they attended a dip tank session on the day of the interview and those who regularly accompany their cattle for grazing. Participation was voluntary and a unique questionnaire identification number was used to maintain participants' confidentiality.

2.2.5 Other data collection

FMD vaccination history was extracted from owner-stock card and animal health technician livestock registers at the time of questionnaire administration. Official veterinary reports from the three wards were retrospectively reviewed to confirm data concerning FMD vaccination.

2.2.6 Statistical analysis

Data were entered into Microsoft Excel. Categorical variables were described with percentages and 95% confidence intervals (CI). Chi-square and Fisher exact tests were used to compare proportions across categorical variables. Continuous variables were described using medians and interquartile ranges (IQR). Kruskal-Wallis (K-W) tests were used to compare continuous variables. Statistical analysis was performed using Epi Info (version 3.5.1 Centre for Disease Control and Prevention, Atlanta, GA, USA) and Minitab (version 16 State College, PA, USA). Results were interpreted at the 5% level of significance.

2.3 Results

2.3.1 Descriptive results

One hundred and four respondents participated. The majority of respondents (73%; 76/104) were owners while the remaining were hired cattle handlers. Eighty-four percent of respondents (87/104) were male. Twenty-six percent (27/104) were single, 55% (57/104) married, 4% (4/104) divorced and 15% (15/104) widowed. The median age of respondents

was 48 (interquartile range: 33-66) years. Twenty-one percent (22/104) of the respondents had no formal education, 38% (40/104) had completed primary education, 36% (37/104) completed secondary education and 1 (1/104) respondent had completed tertiary education.

The median (IQR) number of cattle owned by respondents was 11 (6-19) cattle. The median (IQR) time since the most recent purchase of cattle in the herd was 4 (2-13) years, the median time since the last sale was 1 (1-2) years, the median time since last introduction was 2 years (1.5-2), and the median time in livestock farming was 13.5 (6-73) years. Married households had a median herd size of 8 (interquartile range: 5-15) versus 9.5 (interquartile range: 6-15) for the other categories combined (P = 0.418). The median (IQR) frequency of FMD vaccination reported by respondents was 2 (2-3) times per year. Reported herd vaccination from the owner-stock cards was in complete concordance with animal health technicians' register and the provincial veterinary service data base.

All respondents indicated livestock farming as their major source of income and 11% (11/104) indicated crop farming in addition to livestock. Other raised animals included: 14% (14/104) of respondents reared pigs, 39% (40/104) of respondents reared goats and 74% (77/104) of respondents reared chickens. Three percent (3/104) of respondents indicated using pipe water as a source of livestock drinking water, 82% (85/104) indicated using well water and 18% (19/104) indicated the use of ponds.

Eighty-eight percent (92/104) of respondents indicated that out of all activities undertaken at the dip tank, dipping against ticks and ectoparasites was their favourite. Ninety-six percent of respondents (100/104) reported that they called a veterinarian whenever there is a problem in their herds, while 14% (15/104) indicated self-treatment as an option in addition to contacting a veterinarian.

The highest perceived risk for FMD outbreaks among respondent's cattle was buffalo escape from the park (92%; 96/104), followed by introduction of new animals to the herds (9%; 9/104) and grazing adjacent to park fences (7%; 7/104). Twelve percent (12/104) of respondents reported that they knew of a disease that can cause lesions on the tongue, feet and udder. Nineteen percent (20/104) of respondents reported contacts with wildlife during grazing over their entire farming career. Average daily grazing distance was variable with the majority of respondents (91%; 95/104) reporting a daily average of 1-10km as distance travelled.

2.3.2 Satisfaction with dip tank activities

Seventy-nine percent (82/104) of respondents were very satisfied with the current FMD vaccination programme, 16% (17/104) were little satisfied and 5% (5/104) were not satisfied at all. Disease management strategies and crop production varied based on the satisfaction level (Table 1). The median education level of respondents varied significantly across levels of satisfaction (P = 0.036) with standard 9 (interquartile range: 2-12) for non-satisfied respondents, standard 3 (interquartile range: 0-6) for the little satisfied respondents and standard 7 (interquartile range: 2-11) for very satisfied respondents (Table 2). Non-satisfied respondents were more likely to treat sick animals themselves rather than seek veterinary assistance (P = 0.002).

Table 1. The association between categorical predictor variable and levels of satisfaction with dip tank activities

Variable	Frequency	Not satisfied at all (n=10)		Little satisfied (n=26)		Very satisfied (n=68)		P-value*
			% (95%CI)		% (95%CI)		% (95%CI)	
Status of respondents								
Owner	76	8	80 (48-97)	21	81 (62-93)	47	69 (57-79)	0.456
Gender								
Male	87	9	90 (60-100)	21	81 (62-93)	57	84 (74-91)	0.796
Marital status								
Single	27	3	30 (8-62)	5	19 (7-38)	19	28 (18-39)	0.658
Married	57	6	60 (29-86)	15	58 (38-75)	36	53 (41-65)	0.864
Divorced/Widowed	21	1	10 (0.5-40)	8	31 (15-50)	12	18 (10-28)	0.651

Most important source of income								
Livestock	103	9	90 (60-100)	26	100 (89-0)	68	100 (96-100)	0.090
Crop	11	0	0 (0-26)	6	23 (10-42)	5	7 (3-16)	0.044
Other animals kept								
Pig	13	1	10 (0.5-40)	6	23 (10-42)	6	9 (4-17)	0.168
Goat	40	3	30 (8-62)	11	42 (25-62)	26	38 (27-50)	0.791
Chicken	78	6	60 (29-86)	21	81 (62-93)	51	75 (64-84)	0.435
Source of drinking water								
Pipe	3	0	0 (0-26)	2	8 (1-23)	1	1 (0-7)	0.231
Well	85	8	80 (48-97)	20	77 (58-90)	57	84 (74-91)	0.732
Pond	19	2	20 (4-52)	6	23 (10-42)	11	16 (9-26)	0.732
History of previous FMD outbreaks	12	2	20 (4-52)	2	8 (1-23)	8	12 (6-21)	0.582
Disease management practices								
Contacting a veterinarian	100	7	70 (38-92)	26	100 (89-0)	67	99 (93-100)	0.002

Self-treatment	12	5	50 (21-79)	3	12 (3-29)	4	6 (2-14)	<0.001
Contact with wildlife	20	1	10 (0.5-40)	4	15 (5-33)	15	21 (12-31)	0.563
Grazing adjacent to the Park	53	3	30 (8-62)	13	50 (31-69)	37	54 (43-66)	0.351

*Based on Fisher exact and chi-square tests

CI = Confidence interval

Table 2. Comparison of the level of satisfaction with FMD vaccination across continuous variables for 104 participants

Variable	Not satisfied at all		Little satisfied		Very satisfied		P-value*
	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	
Age of respondents (years)	8	31 (25-53)	22	56 (36-67)	59	45 (33-67)	0.065
Level of education	10	9 (2-12)	24	3 (0-6)	66	7 (2-11)	0.036
Time since last purchase of cattle (years)	2	2	7	4 (2-11)	21	4 (2-19)	0.407
Time since last sale of cattle (years)	5	1 (1-2)	8	1 (1-1)	31	2 (1-2)	0.055
Time since last introduction of new stock (years)	2	2	4	2 (2-3)	7	2 (1-2)	0.272
Duration in livestock farming (years)	5	22 (9-40)	13	17 (8-37)	42	12 (5-23)	0.487
Daily grazing distance (km)	10	3 (2-5)	26	4 (2-5)	68	4 (2-5)	0.635
Number of cattle owned by respondents	10	11 (6-27)	26	7 (6-19)	68	12 (5-19)	0.639
Number of herds owned by respondents	10	1 (1-1)	26	1 (1-1)	68	1 (1-1)	0.866
Frequency of annual FMD vaccination	10	2 (2-2)	26	2 (2-3)	68	2 (2-3)	0.666

*Based on Kruskal-Wallis tests

IQR = Interquartile range

Table 3. Frequency of responses to questions that could affect farmers' participation in vaccination

Questions	Yes			No			Unsure		
	N	(%)	Score	N	(%)	Score	N	(%)	Score
Vaccination can reduce disease in cattle	94	90	1	2	2	-1	8	8	0
Vaccination can make cattle sick	10	10	-1	75	72	1	19	18	0
Vaccination can cause abortion in cattle	15	14	-1	78	75	1	11	11	0
Vaccination improves cattle wellbeing	96	92	1	6	6	-1	2	2	0
Vaccination decreases feed intake in cattle	4	4	-1	94	90	1	6	6	0
Should sick cattle be presented for vaccination?	102	98	1	2	2	-1			
Should pregnant cattle be presented for vaccination?	36	35	1	67	64	-1			

2.3.3 Vaccination perception score

The majority of respondents had favourable perceptions to vaccination (Table 3), however, 64% (67/103) believed that pregnant animals should not be presented for vaccination. The vaccination perception score of respondents varied over level of satisfaction with the dip tank activities ($P<0.001$) and the median (IQR) was -0.5 (-2 – 0) for the non-satisfied, 3 (2 – 4) for the little satisfied and 5 (5 – 7) for the very satisfied respondents. The median (IQR) vaccination perception score was 5 (2.7 – 5) for the non-formal education level, 5 (4 – 7) for primary level education and 5 (3.7 – 7) for the secondary level education but the difference was not significant ($P = 0.201$).

2.4 Discussion

The aim of this study was to determine the perceptions of communal farmers concerning FMD epidemiology and control at a single location of the wildlife/livestock interface of the Kruger National Park. To our knowledge, this is the first farmer based survey regarding the perceptions of communal farmers on FMD control since the development of the Great Limpopo Transfrontier Conservation Areas (GLTFCA).

More respondents (76/104) were involved in herding their own cattle rather than employing paid handlers. Both men and women were involved in herding animals in this area with men accounting for 84% (87/104) of the respondents. In communal areas of South Africa, men and women share the responsibility of keeping livestock (Bester, et al., 2009). Communal farmers have been known to keep cattle for socio-cultural purposes including Lobola (compensation to the family of the bride prior to a wedding ceremony) and to settle disputes (compensation for damages) in communal areas (Chimonyo, et al., 1999).

The majority of farmers (64%) indicated that pregnant animals should not be vaccinated and this is a possible factor that would limit participation in a vaccination campaign. Sick animals, however, were not perceived as a reason to not present animals for vaccination. No other evaluated factors were perceived to affect farmers' presentation of cattle for vaccination. Livestock are important to communal farmers for special ceremonial gatherings such as marriage feasts, weddings, funerals and circumcision (Bayer, et al., 2004) suggesting that married households might have larger herd sizes. Although not statistically significant, married households actually had descriptively smaller herd sizes when compared to other categories.

Seventy-four percent of the respondents had either primary or secondary education qualifications indicating that the majority of farmers are literate and therefore more likely to adopt innovations. However, the majority of non-satisfied individuals had high education

qualifications. This suggests that more educated farmers perceived inadequacies in the current animal health programmes and more education was descriptively associated with large vaccination perception scores.

FMD is considered the most important livestock disease at the livestock wildlife interface (Vosloo, et al., 2002b; Vosloo, et al., 2002a; Thomson and Bastos, 2004), yet farmers do not have extensive knowledge of the disease. In this study, only 12% of the respondents indicated knowledge of any disease causing lesions similar to FMD when described in the local language. This suggests that despite the fact that efforts are in place for the control of FMD at the livestock wildlife interface few farmers have adequate knowledge. Therefore, there is a need for educational programmes concerning FMD and other important livestock diseases among communal farmers in addition to the current programmes. The high number of respondents (96%) indicating that they contact a veterinarian for disease situations is a reflection of animal health awareness within the study community and veterinarians could be an important source of educational material.

With the global increase in the human population, there is a need to improve livestock production across the entire livestock industry. Beef is in high demand for export markets on the basis of taste and texture (Delgado, et al., 1999; Chadwick, et al., 2008). However, some areas in Africa do not have sufficient beef to feed the local populations (Albrechtsen, et al., 2005). Other animals raised in addition to cattle include pigs, goats, and chickens. Goats are herded together with cattle in many communal areas of South Africa (Bester, et al., 2009) and are not routinely vaccinated against FMD. The presence of small ruminants could therefore be a risk factor for the occurrence or extended propagation of outbreaks. Respondents indicated that wildlife increase the risk for disease in livestock at water points and shared grazing. Furthermore, the African buffalo was reported by the majority of respondents (92%) as representing a risk for disease outbreaks in cattle. Contacts between livestock and wildlife

reservoirs have been previously reported to occur at this interface (Brahmbhatt, et al., 2012). These findings indicate that some knowledge concerning FMD epidemiology has been transferred to the local community.

This study is limited to information obtained from livestock owners and handlers using an in-person questionnaire and did not collect information related to the economic aspects of FMD vaccination and control. Another limitation is that farmers might not have presented their true views because of the nature of the in-person interview. Selection bias might have also occurred based on the convenient selection of respondents for the interview. Future studies should include the views of all stakeholders (veterinarians and animal health care workers) involved in vaccine administration.

The perception of livestock owners concerning a disease control intervention (e.g. FMD vaccination) is critical because this perception affects their decision to adopt a new technology or innovation (Adesina and Baidu-Forson, 1995). The current findings might therefore be useful in planning and implementing the progressive control of FMD and other diseases at the wildlife/livestock interface of the Great Limpopo Trans-frontier Parks.

3 HERD IMMUNITY STATUS OF FOOT-AND-MOUTH DISEASE VACCINATION IN CATTLE

3.1 Introduction

Foot-and-mouth disease (FMD) is an economically important disease of livestock in the tropics (Tanya, et al., 2003) and the disease is considered endemic in much of sub-Saharan Africa (Vosloo, et al., 2002b; Jori, et al., 2009). In South Africa, FMD is endemic in the Kruger National Park (KNP) due to the presence of African buffaloes (*Syncerus caffer*) and hence surrounding areas have been classified as FMD protection zones with vaccination (Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health, 2012). All three South African Territories serotypes (SAT 1, SAT 2 and SAT 3) of the FMD viruses have been identified in African buffaloes in the KNP and adjacent nature reserves (Vosloo, et al., 1995; Vosloo, et al., 2002b; Thomson, et al., 2003). African buffaloes act as FMDV carriers and have been associated with outbreaks in impala (*Aepycerus melampus*) within KNP and in cattle within the bordering communal areas (Thomson and Bastos, 2004; Vosloo, et al., 2009).

Cattle at the interface with KNP are routinely vaccinated using a trivalent vaccine containing all three SAT serotypes of FMDV. Based on an assumed basic reproduction number of 4 for FMD, 75% of the cattle population should be immunised (vaccinated and developed sufficient neutralising antibodies) to achieve herd immunity and prevent FMD virus transmission.

FMD viruses exhibit extensive intratypic antigenic variability so that a vaccine prepared from one isolate will not necessarily provide protection against infection with another field virus of the same serotype (Fargeaud, 1995). There is a need to match field viruses to the available reference strains to select a vaccine with expected utility within the target region. FMD control programmes should utilise vaccines developed from representative field isolates to

adequately prevent outbreaks (Paton, et al., 2005). The performance of vaccines containing FMDV serotypes O, A and Asia 1 has been studied extensively elsewhere (Pay and Hingley, 1987; Guo, et al., 2005; Eblé, et al., 2006; Chen and Liu, 2013; Lee, et al., 2013), however, it has been demonstrated that the alhydrogel-saponin SAT type vaccine preparations performed less than the oil adjuvanted preparations under field situation in South Africa (Hunter, 1996).

Chemically inactivated FMD vaccines induce a short-lived duration of immunity similar to other inactivated vaccines (Hunter, 1998). Therefore, vaccine manufacturers typically recommend that cattle in an endemic setting are revaccinated after an initial double primary course at least three times a year (Woolhouse, et al., 1996).

The objective of this study was to estimate the proportion of cattle with presumed protective titres for FMD and herds with adequate herd immunity in the Mnisi Community, Bushbuckridge, Mpumalanga Province South Africa.

3.2 Materials and methods

3.2.1 Ethical Clearance

The study was approved by the Animal Ethics Committee (Project Number V010-12) of the Faculty of Veterinary Science at the University of Pretoria and Section 20 (Animal Disease Act) approval was obtained from the Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health (Application Number 12/11/1/1) of the Republic of South Africa.

3.2.2 Study location

Mnisi Community is a communal area adjacent to KNP in the FMD protection zone of the Mpumalanga Province of South Africa. The control zone consists of game parks and KNP that are infected and the protection zone is divided into zones with and without vaccination of cattle (Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health, 2012). Cattle in the FMD protection zone with vaccination are routinely vaccinated using a trivalent vaccine (SAT 1, SAT 2 & SAT 3; Aftovax® Merial Animal Health Ltd, Botswana Vaccine

Institute Gaborone). In addition to vaccination, game-proof fences are maintained at the western borders of the KNP and adjoining game parks to prevent contacts between livestock and infected wildlife reservoirs. Weekly clinical surveillance is conducted in the protection zone with vaccination, within which the Mnisi Community dip tanks fall.

FMD outbreaks have been reported in the protection zones of the Limpopo and Mpumalanga provinces and the KNP since 2008. Serotype SAT 1 was responsible for 4 outbreaks in 2009, 4 outbreaks in 2010 and 45 subsequent reports of propagating outbreaks in 2011. The 2011 outbreaks occurred in disease free zones of KwaZulu Natal and Gauteng provinces. SAT 2 was responsible for 2 outbreaks in 2008, 4 outbreaks in 2010, 2 outbreaks in 2011 and 5 outbreaks in 2012. SAT 3 was responsible for 1 outbreak in KNP wildlife in 2008 (WAHID, 2013).

3.2.3 Study design

A cross-sectional cluster sampling of cattle by herd was implemented. Sampling was conducted during May to June 2012 in the 15 community dip tanks of the Mnisi area (Fig 2). Two herds were selected at each dip tank using a list of farmers provided by animal health technicians and within each herd ten cattle (or the entire herd when <10) were selected. The sample size was calculated to estimate the expected herd-level seropositivity (herd with ≥80% seropositive animals) with a 20% absolute error and at the 95% level of confidence. The calculated sample size was 24 herds; however 30 herds were selected to allow for the enrolment of two herds per dip tank. All cattle at least 6 months of age (eligible for vaccination at the previous vaccination session) were sampled.

3.2.4 Specimen collection

Whole blood samples were collected from the mid-coccygeal or jugular vein into 10 ml vacutainer® tubes using Precision glide® needles (Becton, Dickinson and company, Franklin Lakes, New Jersey, USA). Blood was allowed to clot at ambient temperature in the field and

transported to the laboratory within 6 hours of collection. Blood samples were centrifuged in the laboratory at 1450 g for 10 minutes. Serum was decanted into sterile cryovials and stored at -20°C until testing. Sera were packaged according to the Regulations of the National Road Traffic Act, 1996 (Act No. 93 of 1996) of the Republic of South Africa and transported under the necessary movement permit on ice to the Transboundary Animal Diseases Programme Laboratory of the Onderstepoort Veterinary Institute (TADP), Pretoria, for testing.

3.2.5 Serological testing for FMDV-specific antibodies

Serum samples were tested for antibodies against FMDV structural proteins using liquid-phase blocking ELISA (Hamblin, et al., 1986b) employing TADP developed reagents for SAT 1, SAT 2 and SAT 3. Briefly, ELISA plates were coated with 50µl/well rabbit antisera specific for the FMD virus 146S antigen and incubated for 1 hour at 37°C. After incubation, test plates were washed three times with phosphate buffered saline (pH 7.4) and blot dried. In U-bottomed multiwell plates (carrier plates) 50µl of a duplicate, twofold series of each test serum was prepared, starting at 1/20. 50µl of FMD virus 146S viral antigen was added to each well and plates were incubated for 1 hour at 37°C. The addition of the antigen increased the final serum dilution to 1/40. 50µl of 146S antigen/serum mixture was transferred from the carrier plates to wells of the rabbit serum-coated ELISA plates and incubated for 1 hour at 37°C on an orbital shaker and then washed and blot dried. 50µl of guinea pig antiserum against the 146S viral antigen used in the previous step and pre-blocked with normal bovine serum diluted in washing buffer-tween$_{20}$ was added to the plate and incubated at 37°C for 1 hour on an orbital shaker and subsequently washed. 50µl of the rabbit anti-guinea pig immunoglobulin conjugated to horseradish peroxidase was added to all wells of the plate and incubated for 1 hour at 37°C on an orbital shaker. Plates were washed three times and blot dried. 100µl of substrate solution containing 0.05%H_2O_2 + Tetramethylbenzidine (TMB) was added to all wells of the plate and incubated at room temperature for 15 minutes. The reaction

was stopped after 15 minutes by the addition of 50µl of 1 M sulphuric acid. Plates were read at 450 nm on a spectrophotometer linked to a computer and antibody titres were expressed as the 50% end-point titre, i.e. dilution at which the optical density of test sera was 50% of the mean optical density of the antigen control wells (Kärber method).

3.2.6 Data analysis

Animal identification, sex, age, breed, vaccination date, sampling date, and the antibody titre for each animal in the study were entered into a spread sheet. Cattle were categorised as seropositive if the ELISA titre was 1.6 $\log_{10}$ or greater for each virus serotype. The percentage of seropositive cattle per herd was determined. Categorical data were described with percentages and 95% confidence intervals (CI) and continuous data were described using medians and interquartile ranges (IQR). Chi-square and Fisher's exact tests were used to compare proportions across categorical variables and Kruskal-Wallis (KW) tests were used to compare factors for quantitative (non-normal) data. Significance was set as $P<0.05$. Descriptive data analysis was performed with EpInfo™ (Centre for Disease Control and Prevention, Alanta, GA, USA) and Open Epi (Open Source Epidemiologic Statistics for Public Health), version 2.3.1, www.OpenEpi.com. Commercially available software (IBM SPSS Statistics Version 21, International Business Machines Corp., Armonk, New York, USA) was used to estimate the seroprevalence while adjusting for clustered sampling and the different population size of cattle at each dip tank.

3.3 Results

A total of 286 blood samples were collected from 2 herds each in 15 community dip tanks within the study area. The median (IQR) age for all the animals sampled was 4.5 (2.5-6.0) years and the median (IQR) period since most recent FMD vaccination was 189 (168-241) days. Relative to an antibody titre of ≥1.6 $\log_{10}$, estimated seroprevalence was 20% (95%CI: 14-26), 39% (95%CI: 32-46) and 22% (95%CI: 17-27) to SAT 1-3, respectively. Median

titres for each SAT serotype varied among herds and dip tanks (Figs 3-5). Overall, only 4%, 15% and 9% of the cattle had antibody titre ≥2 $\log_{10}$ to SAT 1, SAT 2 and SAT 3 respectively. Seropositivity was less than 80% for all SAT serotypes in all but a single herd (Tables 4 and 5). One herd in Share Community had a markedly higher serological response with 80% proportions of cattle being seropositive for SAT 3. Herds in Share and Utha Scheme also had 50% and 60% of cattle seropositive for SAT 3 respectively. A retrospective review of the records of the previous mass vaccination campaign across the study area indicated high vaccination coverage, and therefore low titres indicate poor serological responses to vaccination.

Eighteen percent (95%CI: 10 - 29) of male and 21% (95%CI: 16 - 27) of female cattle were seropositive for SAT 1 (Table 6; P = 0.575). Seropositivity was highest in animals older than 2 years, 22% (95%CI: 17 - 28) but age was not a significant predictor of SAT 1 serological status (P = 0.125). Brahman cattle had significantly lower SAT 1 seropositivity compared to Brahman cross and the local Nguni breed (P = 0.033).

Forty percent, (95%CI: 29 - 53) of male and 38% (95%CI: 31 - 44) of female cattle were seropositive for SAT 2 (P = 0.686). Seropositivity was highest in animals older than 2 years, 40% (95%CI: 34 - 46), but the effect of age was not significant (P = 0.143).

Eighteen percent (95%CI: 10 – 29) of male and 25% (95%CI: 20 – 31) of female cattle were seropositive for serotypes SAT 3 (P = 0.208). Seropositivity was highest in animals less than or equal to 1 year, 28% (95%CI: 11 – 51), but age was not a significant predictor of SAT 3 serological status (P = 0.117).

Table 4. Distribution of herd immunity to FMD vaccination at titre ≥1.6 $\log_{10}$

					Percentages		
Dip tank	**Vaccination coverage (%)**	**#**	**Vaccination period**	**N**	**SAT 1 % (95%CI)**	**SAT 2 % (95%CI)**	**SAT 3 % (95%CI)**
Eglington	0	1	281	10	0 (0-26)	30 (8-62)	0 (0-26)
		2	281	10	40 (14-71)	70 (38-92)	0 (0-26)
Share	84	1	258	10	50 (21-79)	60 (29-86)	50 (21-79)
		2	258	10	50 (21-79)	60 (29-86)	80 (48-97)
Utha Scheme	97	1	631	10	50 (21-79)	50 (21-79)	60 (29-86)
		2	631	10	0 (0-26)	40 (14-71)	30 (8-62)
Shorty	86	1	329	7	0 (0-26)	29 (5-67)	14 (1-53)
		2	329	10	30 (8-62)	30 (8-62)	30 (8-62)
Clare B	100	1	182	10	30 (8-62)	50 (21-79)	30 (8-62)
		2	182	10	10 (1-40)	30 (8-62)	10 (1-40)
Gottenburg	100	1	161	10	10 (1-40)	10 (1-40)	10 (1-40)
		2	161	10	10 (1-40)	10 (1-40)	10 (1-40)
Clare A	100	1	189	10	20 (4-52)	30 (8-62)	20 (4-52)
		2	189	7	29 (5-67)	43 (12-78)	29 (5-67)
Welverdiend A	100	1	168	10	40 (14-71)	60 (29-86)	40 (14-71)
		2	168	10	20 (4-52)	30 (8-62)	20 (4-52)
Seville B	100	1	161	10	0 (0-26)	50 (21-79)	0 (0-26)
		2	161	6	17 (1-59)	17 (1-59)	17 (1-59)
Welverdiend B	100	1	175	10	30 (8-62)	30 (8-62)	20 (4-52)
		2	175	10	20 (4-52)	20 (4-52)	20 (4-52)

Seville A	99	1	161	8	13	(1-48)	25	(4-61)	25	(4-61)
		2	161	10	20	(4-52)	60	(29-86)	20	(4-52)
Tlhavekisa	100	1	202	10	30	(8-62)	30	(8-62)	30	(8-62)
		2	202	10	20	(4-52)	40	(14-71)	40	(14-71)
Utha A	75	1	192	10	30	(8-62)	40	(14-71)	30	(8-62)
		2	192	10	0	(0-26)	60	(29-86)	10	(1-40)
Hlalakahle	100	1	189	9	11	(1-44)	0	(0-26)	33	(9-67)
		2	189	10	20	(4-52)	30	(8-62)	20	(4-52)
Athol	0	1	357	9	0	(0-26)	56	(24-84)	0	(0-26)
		2	357	10	0	(0-26)	40	(14-71)	10	(1-40)

*Number of days since most recent FMD vaccination

CI = Confidence interval

1 , 2 = Herd order

Vaccination coverage as obtained from the Veterinary Services data base

Table 5. Distribution of herd immunity to FMD vaccination at titre ≥2.0 $\log_{10}$

					Percentages		
Dip tank	**Vaccination coverage (%)**	**#**	**Vaccination Period***	**N**	**SAT 1 % (95%CI)**	**SAT 2 % (95%CI)**	**SAT 3 % (95%CI)**
Eglington	0	1	281	10	0 (0-26)	10 (1-40)	0 (0-26)
		2	281	10	0 (0-26)	30 (8-62)	0 (0-26)
Share	84	1	258	10	30 (8-62)	30 (8-62)	20 (4-52)
		2	258	10	20 (4-52)	30 (8-62)	30 (8-62)
Utha Scheme	97	1	631	10	10 (1-40)	40 (14-71)	10 (1-40)
		2	631	10	0 (0-26)	10 (1-40)	0 (0-26)
Shorty	86	1	329	7	0 (0-35)	29 (5-67)	29 (5-67)
		2	329	10	10 (1-40)	0 (0-26)	20 (4-52)
Clare B	100	1	182	10	10 (1-40)	20 (4-52)	30 (8-62)
		2	182	10	0 (0-26)	0 (0-26)	10 (1-40)
Gottenburg	100	1	161	10	10 (1-40)	10 (1-40)	10 (1-40)
		2	161	10	0 (0-26)	10 (1-40)	10 (1-40)
Clare A	100	1	189	10	0 (0-26)	10 (1-40)	10 (1-40)
		2	189	7	0 (0-35)	14 (1-53)	29 (5-67)
Welverdiend A	100	1	168	10	0 (0-26)	30 (8-62)	10 (1-40)
		2	168	10	0 (0-26)	20 (4-52)	0 (0-26)
Seville B	100	1	161	10	0 (0-26)	10 (1-40)	0 (0-26)
		2	161	6	0 (0-39)	0 (0-39)	0 (0-39)
Welverdiend B	100	1	175	10	0 (0-26)	0 (0-26)	0 (0-62)
		2	175	10	0 (0-26)	10 (1-40)	10 (1-40)
Seville A	99	1	161	8	0 (0-31)	13 (1-48)	0 (0-31)

		2	161	10	0	(0-26)	10	(1-40)	10	(1-40)
Tlhavekisa	100	1	202	10	20	(4-52)	10	(1-40)	20	(4-52)
		2	202	10	0	(0-26)	10	(1-40)	10	(1-40)
Utha A	75	1	192	10	10	(1-40)	20	(4-52)	10	(1-40)
		2	192	10	0	(0-26)	20	(4-52)	0	(0-26)
Hlalakahle	100	1	189	9	0	(0-28)	0	(0-28)	0	(0-28)
		2	189	10	0	(0-26)	20	(4-52)	0	(0-26)
Athol	0	1	357	9	0	(0-28)	0	(0-28)	0	(0-28)
		2	357	10	0	(0-26)	10	(1-40)	0	(0-26)

*Number of days since most recent FMD vaccination

CI = Confidence interval

1 , 2 = Herd order

Vaccination coverage as obtained from the Veterinary Services data base

Table 6. Serological responses to SAT 1, SAT 2 and SAT 3 on the basis of sex, age and breed (titre ≥1.6 $\log_{10}$)

	N	Sample positive	Sample negative	Proportion	95%CI	P-value
SAT 1	286	58	228	20	14 – 26	
Sex						
Male	62	11	51	18	10 – 29	0.575
Female	224	47	177	21	16 – 27	
Age						
≤12 months	18	4	14	22	7 – 45	0.125
13-24 months	38	3	35	8	2 – 20	
>24 months	230	51	179	22	17 – 28	
Breed						
Brahman	46	4	42	9	2 – 20	0.033
Typical						
Brahman cross	108	24	84	22	15 – 31	
&						
Nguni	132	30	102	23	16 – 30	
SAT 2	286	109	177	39	32 – 46	
Sex						
Male	62	25	37	40	29 – 53	0.686
Female	224	84	140	38	31 – 44	
Age						
≤12 months	18	3	15	17	4 – 39	0.143

13-24 months	38	14	24	37	23 – 53	
>24 months	230	92	138	40	34 – 46	
Breed						
Brahman	46	14	32	30	18 – 45	0.242
Typical						
Brahman cross	108	37	71	34	26 – 44	
&						
Nguni	132	58	74	44	36 – 52	
SAT 3	286	68	218	22	17 – 27	
Sex						
Male	62	11	51	18	10 – 29	0.208
Female	224	57	167	25	20 – 31	
Age						
≤12 months	18	5	13	28	11 – 51	0.117
13-24 months	38	4	34	11	3 – 23	
>24 months	230	59	171	26	20 – 32	
Breed						
Brahman	46	5	41	11	4 – 22	0.025
Typical						
Brahman cross	108	26	82	24	17 – 33	
&						
Nguni	132	37	95	28	21 – 36	

CI = Confidence interval

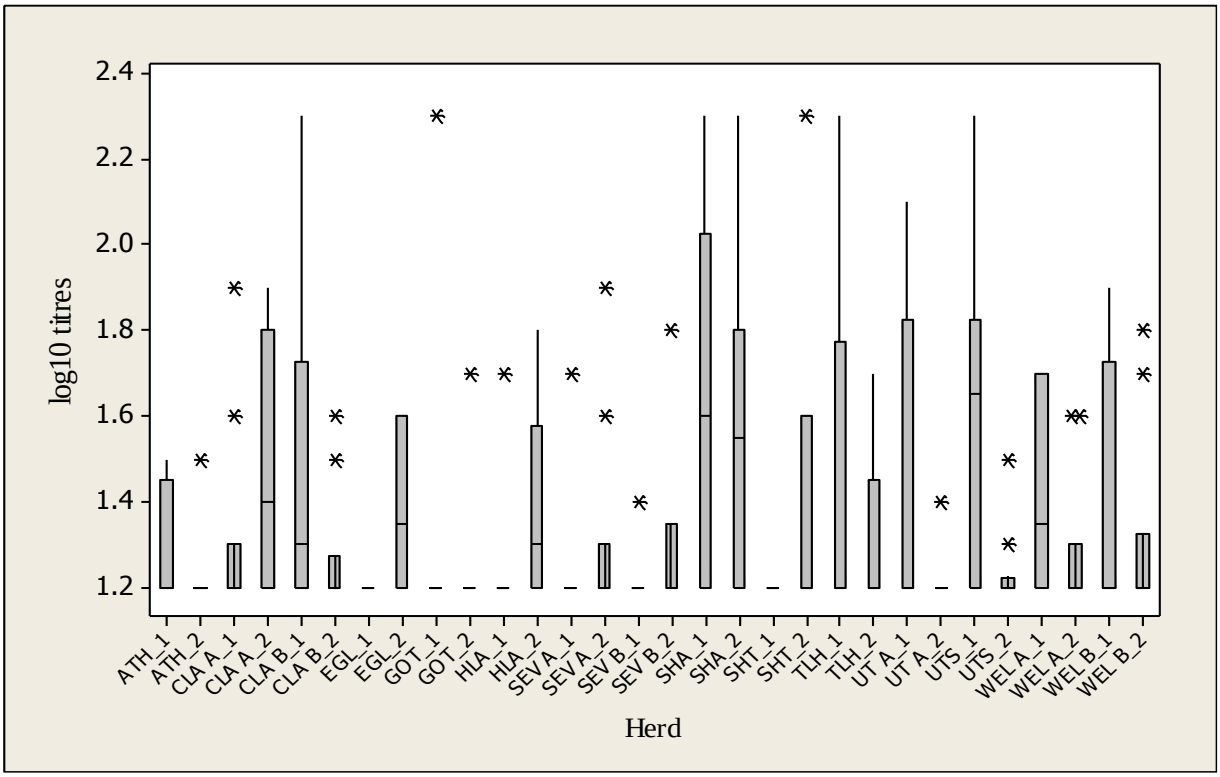

Figure 3. SAT 1 titre distributions by herd

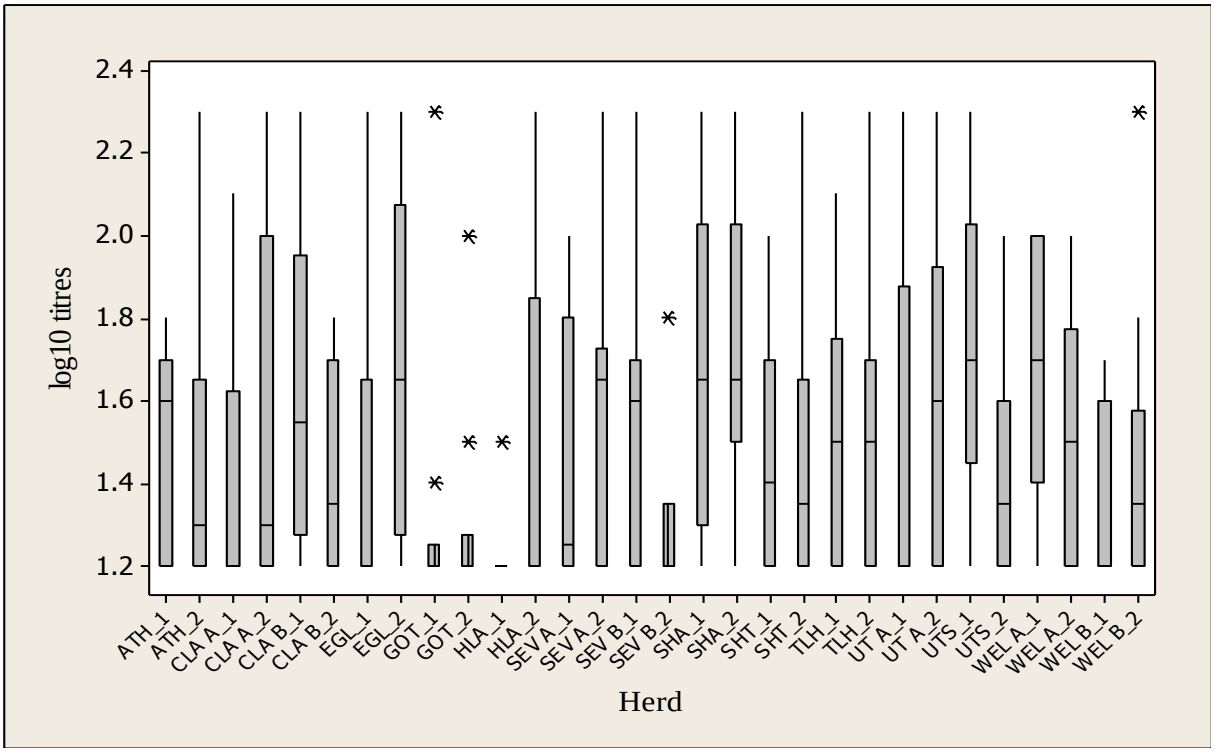

Figure 4. SAT 2 titre distributions by herd

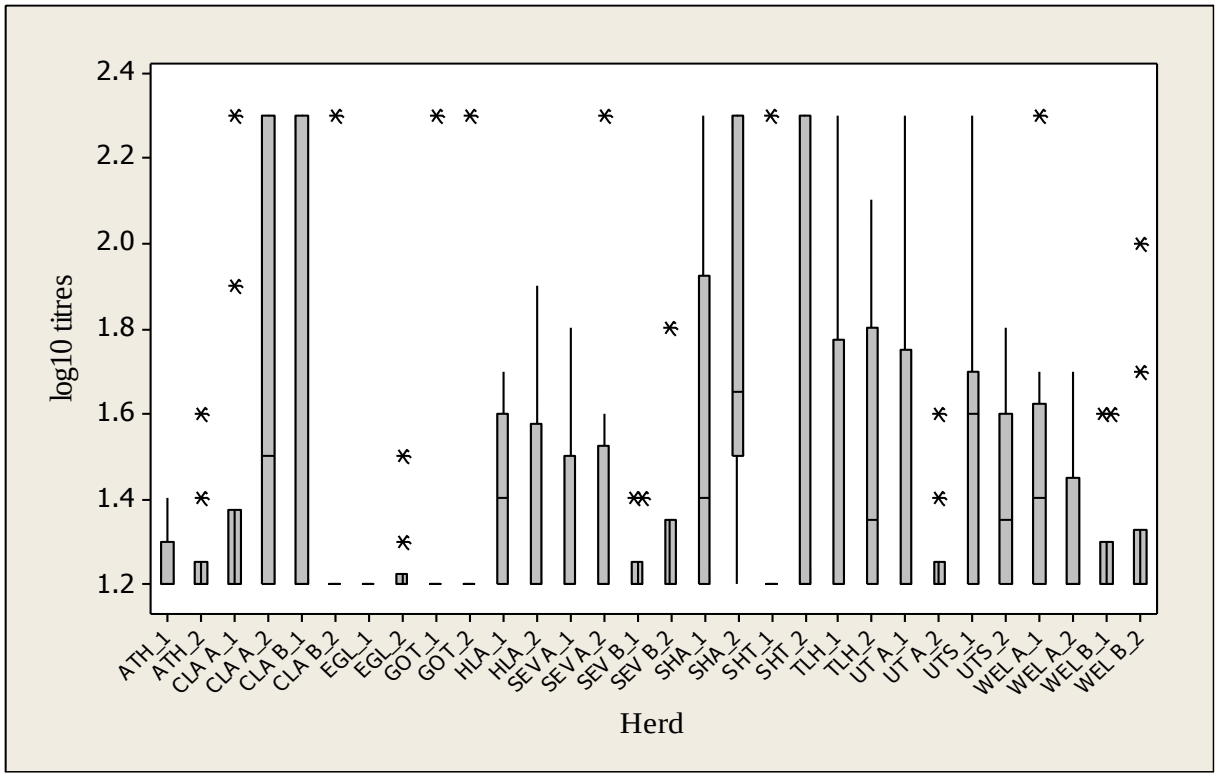

Figure 5. SAT 3 titre distributions by herd

3.4 Discussion

Cattle in the Mnisi communal area are routinely vaccinated using an inactivated FMD vaccine containing SAT 1, SAT 2 and SAT 3 serotypes. However, the proportion of cattle with high levels of detectable antibody was low suggesting that the area is at risk for the active spread of FMD virus. This finding is consistent with previous research where the antibody level induced by alhydrogel-saponin SAT type vaccine preparation fell below 1.6$\log_{10}$ VNT titre between 2 and 3 months after inoculation (Hunter, 1996). However, in another study using a trivalent double emulsion vaccine, antibody levels to all SAT serotypes were maintained at >1.6 $\log_{10}$ for eleven months post-vaccination (Hunter, 1996).

In this study, we used heterologous antigens to test for FMD-specific antibodies in sera from vaccinated cattle using liquid-phase blocking ELISA. This might be an explanation for the high variability in measured titres observed for SAT 1 and SAT 3. Since cattle in the protection zones are routinely vaccinated for FMD and all animals were older than 6 months of age, it was assumed that all sampled cattle had received at least a single vaccination prior to the study.

The majority of sampled cattle were females and the SAT 1 antibody response was descriptively greater than in sampled bulls. Female cattle form the majority of the cattle population within communal areas. Farmers might also present more female cattle for vaccination because bulls might be too difficult to handle at the dip tanks. Serotype SAT 2 antibody titres appeared to be more consistent relative to the SAT 1 and SAT 3 titres which might suggest a closer antigenic relationship between the vaccine strain and the test antigen. This could also explain why more herds had seropositive proportions greater than 50% for SAT 2 antibodies compared to the other serotypes. SAT 2 viruses have been reported to have more sequence variation in the VP1 gene relative to other serotypes and vaccine manufacturers often select immunodominant vaccine strains with broad antigenic coverage

(Rweyemamu, 1978). The antibody response to SAT 3 was relatively poor in adult cattle and demonstrated high variability between herds and dip tanks (similar to SAT 1). However, there were two herds at Utha Scheme that had 50% and 60% seropositivity and a herd at Share with 80% seropositivity. Although no clinical signs of FMD were reported in these locations during the study, evidence of high antibodies to SAT 3 may suggest the undetected virus circulation in the cattle populations as reported earlier (Jori, et al., 2014), which may have a serious implication for control.

Age was not a significant predictor for seropositivity contrary to our expectations that older cattle would have large seropositive proportions relative to younger calves because of exposures to repeated immunisations. This could be due to rapid antibody decline and poor stimulation of memory B-cells. Sex was not a significant predictor for any of the SAT type FMD virus but breed appeared to be important for SAT 1 and SAT 3. Brahman cattle had less seropositivity for SAT 1 and SAT 3 and the local Nguni breed might have better immune response to vaccination. The genetic makers of the indigenous Nguni breeds in terms of disease resistance and productivity have been reviewed elsewhere (Scholtz, 1988; Mapiye, et al., 2007).

FMD vaccines predominantly stimulate a humoral immune response in cattle and there is a strong correlation between antibody levels and protection against challenge with homologous virus (Ahl, et al., 1983; Sutmöller, et al., 1983; Pay and Hingley, 1987). Therefore serological evidence of FMD antibodies in vaccinated animals in the absence of circulating field virus is an indicator of protection to field challenge. The low proportion of seropositivity observed might be an indication of rapidly declining humoral responses and reduced protection, which is consistent with reports that aqueous FMD vaccines stimulate a short-lived duration of immunity (Hunter, 1998).

This study is limited by the fact that the cattle were selected based on the convenient sampling of farmers at dip tanks and might therefore not be completely representative of the target population. The incomplete sampling of cattle within the herd is also another limitation that affects inferences concerning the proportion of cattle seropositive within each herd. The use of heterologous antigens within the liquid-phase blocking ELISA might have underestimated the proportion of cattle classified as seropositive.

Owing to the increased occurrence of outbreaks in recent time despite sustained efforts in routine prophylactic vaccination of cattle at the interface, there is a need to develop a cost effective vaccination regimen that will induce effective herd immunity. Post-vaccination monitoring based on serological evaluation of herd immunity should form an integral part of all FMD vaccination programmes within southern Africa.

4 SEROLOGICAL RESPONSES AND DURATION OF FMD TITRES IN CATTLE INOCULATED WITH AN INACTIVATED TRIVALENT FMD VACCINE

4.1 Introduction

Foot-and-mouth disease (FMD) causes large economic effects on trade at the local, national and international levels and substantial funds are invested worldwide for prevention and control (OIE, 2012c). Routine vaccination of susceptible cloven hoofed livestock with inactivated FMD vaccine is recommended for control in endemic countries. However, in FMD free countries where the disease has been eradicated, the strategies for control include the use of emergency vaccination with high potency vaccines >6PD_{50}, (six-times the dose of vaccine that protects 50% of the animals challenged) along with the culling of infected animals (Parida, 2009; OIE, 2012a). The OIE classifies FMD vaccines as either "standard" or "high potency" vaccines based on the quantity of antigen. A standard vaccine with a potency of 3PD_{50} and appropriate adjuvant is considered suitable for routine vaccination campaigns in FMD endemic locations (Elnekave, et al., 2013). FMD vaccine potency testing is performed by experimentally infecting vaccinated cattle. The in vivo 50% Protective Dose (PD_{50}) test is the prescribed standard European procedure for the quality control of FMD vaccines (OIE, 2012a). FMD vaccines in endemic countries often contain more than one virus serotype, depending upon the epidemiological situation of the particular country. In southern Africa, the employed vaccines are typically trivalent (SAT 1-3) or bivalent (SAT 1 & 2) depending on the country (Thomson and Bastos, 2004).

Serological assays including virus neutralisation tests (VNT) and enzyme-linked immunosorbent assay (ELISA) have been employed to measure serological responses to vaccination in cattle (Sutmoller and Vieira, 1980; McCullough, et al., 1992b). There is good correlation between antibody titres and protection to challenge with a live virus (Mackowiak,

et al., 1959; Mackowiak, et al., 1962; Pay and Hingley, 1987) but ELISA results have been reported to be more predictive of protection compared to serum neutralisation titres (van Maanen, 1988; van Maanen and Terpstra, 1989; McCullough, et al., 1992b). FMD vaccines frequently applied for prophylactic use in endemic settings provide immunity for a period of 4-6 months in the absence of regular booster doses (Doel, 2003). However, despite a good correlation between serum antibody titres and protection, there are instances where animals with substantial antibody titres are not protected from disease after experimental challenge (McCullough, et al., 1992a). Similarly, animals with low or no detectable antibody do not always succumb to disease (Sobrino, et al., 2001). Therefore, cell-mediated immunity might also be important to confer protection from disease after exposure to FMD virus (Oh, et al., 2012; Carr, et al., 2013).

The objective of this study was to determine the serological responses and the duration of humoral immune response conferred by the current FMD vaccination programme in cattle within the Mnisi Community.

4.2 Materials and methods

4.2.1 Ethical clearance

The study was approved by the Animal Ethics Committee (Project Number V010-12) at the University of Pretoria, Faculty of Veterinary Science. Section 20 approval (Animal Disease Act) was obtained from the Department of Agriculture, Forestry and Fisheries, Directorate: Animal Health (Application Number 12/11/1/1) of the Republic of South Africa.

4.2.2 Description of the study area

Mnisi Community is a communal area situated within the FMD protection zone adjacent to the Kruger National Park (KNP) in Mpumalanga Province of the Republic of South Africa. One of the major activities of the residents of this community is livestock herding that typically employs an extensive free range system. Cattle in this area are routinely vaccinated for FMD virus using a trivalent vaccine containing SAT 1, SAT 2 and SAT 3 antigens.

4.2.3 Sample size justification

The sample size calculations were performed to estimate the proportion of cattle with ≥1.6 $\log_{10}$ titres (seropositive) at any sampling period post-vaccination. It was assumed that 80% of cattle would become seropositive and it was desired to estimate this proportion +/- 10% at the 95% level of confidence. A design effect of 4 was assumed to account for the clustering of cattle within dip tanks and also within herds. The sample size was estimated as 246 cattle based on these assumptions.

4.2.4 Selection of cattle

Four community dip tanks from a list of 16 communities in the Mnisi communal area were purposively selected based on the scheduling of weekly livestock inspection. The four dip tanks were selected to represent 2 dip tanks each from two wards managed by different animal health technicians. At each dip tank, seven herds were conveniently selected after obtaining informed consent from the farmers concerning the necessary 4 month follow-up. Ten cattle older than 6 months of age were purposely selected from each participating herd. The age of enrolled cattle was determined based on dentition and available information from the herder and subsequently categorised as 6-12 months, 13-24 months and >24 months. Selected cattle were ear-tagged for identification.

4.2.5 Vaccination procedures

Provincial government veterinary services performed the routine FMD mass vaccination programme during June 2012. Cattle were vaccinated subcutaneously in the neck region using an automated syringe system. Each animal was injected with 5 ml of a commercial aqueous aluminium hydroxide and saponin-adjuvanted inactivated trivalent FMD vaccine containing SAT 1, SAT 2 and SAT 3 strains (Aftovax®, Merial Animal Health Ltd/Botswana Vaccine Institute Gaborone). The vaccine batch number was 13309 and had a December 2012 expiry date.

4.2.6 Sample collection

Blood samples were collected on the day of vaccination (day 0) and at 2-week intervals over a 4-month follow-up period (days 0 - 112). Whole blood samples were collected from the mid-coccygeal or jugular vein into 10 ml plain evacuated tubes. Blood was allowed to clot at ambient temperature in the field and transported to the laboratory within 6 hours of collection. Blood was centrifuged at 1450 g for 10 min immediately after delivery to the laboratory. Serum was decanted into sterile cryovials and stored at -20˚C until testing. Sera were packaged according to the Regulations of the National Road Traffic Act, 1996 (Act No. 93 of 1996) of the Republic of South Africa and transported on ice to the Transboundary Animal Disease Programme Laboratory of the Onderstepoort Veterinary Institute (TADP), Pretoria for testing.

4.2.7 Laboratory testing

Samples were analysed for FMD-specific antibodies using a liquid phase blocking ELISA as previously described by Hamblin, et al. (1986b). Assays were performed using an in-house developed ELISA kit for SAT 1, SAT 2 and SAT 3. This test is based upon serotype specific blocking of liquid phase FMD heterologous antigen by antibodies in the test serum sample. Antibody titres were expressed as the 50% end-point titres and sera with titres ≥ 1.6 $\log_{10}$ were classified as seropositive.

4.2.8 Statistical analysis

Animal identification, sex, age, breed, vaccination date and antibody titre for each round of testing were entered into a spread sheet. The percentage of seropositive cattle at each round of bleeding was determined and 95% confidence intervals (CI) were calculated using the exact mid-P method. Quantitative data were described using medians and interquartile ranges (IQR). Kruskal-Wallis (KW) tests were used to compare titre data over groups of potential predictors. A linear mixed model was fit to estimate the effect of covariates on measured titres. Models included random effects for dip tank and herd and evaluated fixed effect terms for sampling round, serotype, age category, sex, breed, and an interaction between serotype and sampling round. Descriptive data analysis was performed using one statistical package (Minitab, Version 16 State College, PA, USA) and the linear mixed model in another (IBM SPSS Statistics Version 21, International Business Machines Corp., Armonk, New York, USA). Results were interpreted at the 5% level of significance.

4.3 Results

A total of 293 cattle were sampled in 4 community dip tanks during the 112 day study period. Complete follow-up was not obtainable for all selected cattle (Table 7). At the commencement of the study, few cattle had evidence of pre-existing antibody responses to the SAT viruses (Table 8). However, 14 days post-vaccination, the proportion of seropositive cattle ($\geq$1.6 $\log_{10}$ titre) to the three SAT type virus varied between 66% - 91% with SAT 2 having the highest proportions (Table 8). Overall, the proportions of cattle with titre $\geq$2 $\log_{10}$ for any of the 3 serotypes varied from a minimum of 39% to a high of 77% per location by 14 days post-vaccination (Table 9). Antibody responses peaked up to 98%, 98% and 65% by 42 days post-vaccination for SAT 1, SAT 3 and SAT 3 respectively until starting to decline at 56 days post-vaccination. By the end of the 112 day follow-up, antibody responses to all

serotypes was less than 40% at all of the study locations. However SAT 1 and SAT 2 had the highest proportions of 36% and 30% per study location respectively.

The median SAT 1 antibody titre at 42 days post vaccination varied by age (P = 0.015; Table 10). At 112 days post-vaccination, female cattle had higher SAT 1 titres (P = 0.002). SAT 2 titres at 14 and 42 days post-vaccination were different across age with cattle 6-12 months of age having lower titres compared to other age groups (P = 0.021 and P = 0.025, respectively; Table 11) respectively. Also, female cattle had higher SAT 2 titres compared to males at 56 and 112 days respectively (P = 0.041 and P = 0.004, respectively). Female cattle had higher SAT 3 titres at 42 days post-vaccination (P = 0.011; Table 12). At 42 days post-vaccination, SAT 3 antibody titres also varied by age, breed and location (P = 0.002, P = 0.033, and P = 0.001, respectively).

Overall, age was a significant predictor of antibody titre (P<0.001) with cattle >13 months of age having higher titres than younger cattle (Table 13). Virus serotype was a predictor of antibody titre with SAT 2 having higher titres (P<0.001) and the duration of antibody responses varied by serotype (P<0.001). The predicted Log_{10} antibody peaks were 1.91 at post-vaccination day 14, 2.19 at day 42, and 2.11 at day 42 for SAT 1, SAT 2, and SAT 3, respectively (Table 14).

Table 7. Number of cattle sampled during the 4 months study period

	Dip tank				**Age**			**Breed**			**Sex**		
Day	A	B	C	D	6-12 mn	13-24 mn	>24 mn	Brahman	Brahman cross	Nguni	Female	Male	Total
0	32	45	0	0	6	29	42	26	24	27	62	15	77
14	63	47	70	44	35	70	119	24	98	102	162	62	224
28	59	44	65	56	33	72	119	30	99	95	156	68	224
42	50	51	71	59	33	73	125	21	108	102	167	64	231
56	65	0	66	53	33	55	96	21	79	84	130	54	184
70	65	38	68	49	29	66	125	26	88	106	162	58	220
84	28	44	0	0	7	26	39	14	21	37	53	19	72
98	34	49	51	66	33	58	109	21	89	90	146	54	200
112	65	54	57	64	36	75	129	31	97	112	180	60	240
Total	461	372	448	391	245	524	903	214	703	755	1,218	454	1,672

A = Eglington, B = Clare A, C = Shorty, D = Tlhavekisa

Table 8. Proportions of cattle with antibody titre ≥1.6 $\log_{10}$ according to study location and FMD serotype

Day	Dip tank	SAT 1 % (95%CI)	SAT 2 % (95%CI)	SAT 3 % (95%CI)
0	Eglington	3 (0 – 14)	36 (22 – 55)	16 (6 – 31)
	Clare A	7 (2 – 17)	47 (33 – 61)	16 (7 – 28)
	Shorty	NA	NA	NA
	Tlhavekisa	NA	NA	NA
14	Eglington	81 (70 – 89)	84 (74 – 92)	73 (61 – 83)
	Clare A	85 (73 – 93)	87 (75 – 95)	70 (56 – 82)
	Shorty	86 (76 – 93)	93 (85 – 97)	89 (79 – 95)
	Tlhavekisa	66 (51 – 79)	91 (80 – 97)	89 (77 – 96)
28	Eglington	51 (38 – 63)	53 (40 – 65)	49 (37 – 62)
	Clare A	45 (31 – 60)	45 (31 – 60)	55 (40 – 69)
	Shorty	63 (51 – 74)	68 (56 – 78)	65 (52 – 75)
	Tlhavekisa	75 (62 – 85)	75 (62 – 85)	70 (57 – 81)
42	Eglington	86 (74 – 94)	100 (94 – 100)	88 (77 – 95)
	Clare A	92 (82 – 97)	100 (94 – 100)	94 (85 – 98)
	Shorty	75 (64 – 84)	86 (76 – 93)	83 (73 – 91)
	Tlhavekisa	61 (48 – 73)	92 (82 – 97)	92 (82 – 97)
56	Eglington	54 (42 – 66)	49 (37 – 61)	38 (27 – 51)
	Clare A	NA	NA	NA
	Shorty	68 (56 – 79)	58 (45 – 69)	47 (35 – 59)
	Tlhavekisa	70 (57 – 81)	66 (53 – 78)	60 (47 – 73)
70	Eglington	26 (17 – 38)	29 (19 – 41)	31 (20 – 42)

	Clare A	26 (14 – 42)	37 (23 – 53)	24 (12 – 39)
	Shorty	25 (16 – 36)	44 (33 – 56)	35 (25 – 47)
	Tlhavekisa	33 (21 – 47)	45 (31 – 59)	47 (33 – 61)
84	Eglington	29 (14 – 47)	25 (12 – 43)	36 (20 – 54)
	Clare A	52 (38 – 67)	45 (31 – 60)	39 (25 – 54)
	Shorty	NA	NA	NA
	Tlhavekisa	NA	NA	NA
98	Eglington	18 (7 – 33)	41 (26 – 58)	35 (21 – 52)
	Clare A	37 (24 – 51)	45 (31 – 59)	41 (28 – 55)
	Shorty	31 (20 – 45)	47 (34 – 61)	41 (28 – 55)
	Tlhavekisa	48 (37 – 60)	53 (41 – 65)	61 (48 – 72)
112	Eglington	52 (40 – 64)	57 (45 – 69)	28 (18 – 39)
	Clare A	46 (33 – 60)	59 (46 – 72)	30 (19 – 43)
	Shorty	53 (40 – 65)	77 (65 – 87)	26 (16 – 39)
	Tlhavekisa	66 (53 – 76)	67 (55 – 78)	42 (31 – 55)

CI = confidence interval. NA = no animals sampled

Table 9. Proportions of cattle with antibody titre ≥2.0 $\log_{10}$ according to study location and FMD serotype

Day	Dip tank	No of cattle sampled	SAT 1 % (95%CI)	SAT 2 % (95%CI)	SAT 3 % (95%CI)
0	Clare A	45	4 (1 - 14)	16 (7 - 28)	7 (2 - 17)
	Eglington	32	0 (0 - 9)	9 (2 - 23)	3 (0 -14)
	Shorty	NA	NA	NA	NA
	Tlhavekisa	NA	NA	NA	NA
14	Clare A	47	57 (43 - 71)	77 (63 - 87)	45 (31 - 59)
	Eglington	63	51 (39 - 63)	67 (54 - 77)	44 (34 - 57)
	Shorty	70	53 (41 - 64)	70 (59 - 80)	63 (51 - 74)
	Tlhavekisa	44	39 (25 - 54)	73 (58 - 84)	66 (51 - 79)
28	Clare A	44	27 (16 - 42)	23 (12 - 37)	27 (16 - 42)
	Eglington	59	24 (14 - 36)	24 (14 - 36)	32 (21 - 45)
	Shorty	65	35 (25 - 48)	35 (25 - 48)	38 (27 - 51)
	Tlhavekisa	56	43 (30 - 56)	29 (18 - 41)	48 (35 - 61)
42	Clare A	51	65 (51 - 77)	98 (91 - 100)	86 (75 - 94)
	Eglington	50	40 (27 - 54)	94 (85 - 98)	58 (44 - 71)
	Shorty	71	52 (41 - 64)	70 (59 - 80)	72 (61 - 81)
	Tlhavekisa	59	37 (25 - 51)	94 (85 - 98)	98 (91 - 100)
56	Clare A	0	0	0	0
	Eglington	65	25 (15 - 36)	34 (23 - 46)	15 (8 - 26)
	Shorty	66	29 (19 - 41)	26 (16 - 37)	18 (10 - 29)
	Tlhavekisa	53	45 (32 - 59)	23 (13 - 35)	32 (21 - 45)
70	Clare A	38	5 (0 - 16)	11 (3 - 23)	16 (7 - 30)

	Eglington	65	2 (0 - 7)	5 (1 - 12)	5 (1 - 12)
	Shorty	68	10 (5 - 19)	15 (8 - 25)	12 (6 - 21)
	Tlhavekisa	49	10 (4 - 21)	4 (1 - 13)	18 (9 - 31)
84	Clare A	44	20 (10 - 34)	18 (9 - 32)	16 (7 - 29)
	Eglington	28	7 (1 - 22)	7 (1 - 22)	11 (3 - 26)
	Shorty	NA	NA	NA	NA
	Tlhavekisa	NA	NA	NA	NA
98	Clare A	49	10 (4 - 21)	16 (8 - 29)	16 (8 - 29)
	Eglington	34	6 (1 - 18)	12 (4 - 26)	12 (4 - 26)
	Shorty	51	10 (4 - 20)	12 (5 - 23)	18 (9 - 30)
	Tlhavekisa	66	18 (10 - 29)	17 (9 - 27)	29 (19 - 41)
112	Clare A	54	11 (5 - 22)	20 (11 - 33)	11 (5 - 22)
	Eglington	65	15 (8 - 26)	22 (13 - 33)	8 (3 - 16)
	Shorty	57	12 (6 - 23)	30 (19 - 43)	5 (1 - 14)
	Tlhavekisa	64	36 (25 - 48)	27 (17 - 38)	17 (9 - 28)

CI = confidence interval. NA = No cattle sampled

Table 10. Median and interquartile range (IQR) log_{10} antibody titre for SAT 1 by animal factors and location

Day	Variable	N	Median (IQR)	P-value
0	**Sex**			
	Female	3	1.90 (1.65 – >2.20)	0.346
	Male	1	>2.20	
	Age			
	6 – 12 months	1	>2.20	0.346
	13 – 24 months			
	>24 months	3	1.92 (1.65 – >2.20)	
	Breed			
	Brahman typical	2	1.98	0.632
	Brahman cross	1	>2.20	
	Nguni	1	1.92	
	Location			
	Clare A	3	>2.20 (1.93 – >2.20)	0.157
	Eglington	1	1.64	
14	**Sex**			
	Female	131	>2.20 (1.91 – >2.20)	0.640
	Male	50	>2.20 (1.76 – >2.20)	
	Age			
	6 – 12 months	23	1.93 (1.75 – >2.20)	0.131
	13 – 24 months	60	>2.20 (1.96 – >2.20)	
	>24 months	98	>2.20 (1.87 – >2.20)	
	Breed			

	Brahman typical	21	>2.20 (1.74 – >2.20)	0.590
	Brahman cross	84	>2.20 (1.93 – >2.20)	
	Nguni	76	>2.20 (1.88 – >2.20)	
	Location			
	Clare A	41	>2.20 (1.88 – >2.20)	0.854
	Eglington	51	>2.20 (1.83 – >2.20)	
	Shorty	60	2.20 (1.92 – >2.20)	
	Tlhavekisa	29	2.20 (1.77 – >2.20)	
28	**Sex**			
	Female	100	>2.20 (1.76 – >2.20)	0.837
	Male	34	>2.20 (1.84 – >2.20)	
	Age			
	6 – 12 months	17	>2.20 (1.87 – >2.20)	0.455
	13 – 24 months	44	>2.20 (1.84 – >2.20)	
	>24 months	73	2.12 (1.77 – >2.20)	
	Breed			
	Brahman typical	17	1.94 (1.70 – >2.20)	0.292
	Brahman cross	65	>2.20 (1.82 – >2.20)	
	Nguni	52	>2.20 (1.86 – >2.20)	
	Location			
	Clare A	21	>2.20 (1.80 – >2.20)	0.507
	Eglington	30	1.93 (1.72 – >2.20)	
	Shorty	41	>2.20 (1.86 – >2.20)	
	Tlhavekisa	42	>2.20 (1.87 – >2.20)	
42	**Sex**			

	Female	136	>2.20 (1.88 – >2.20)	0.191
	Male	44	2.18 (1.80 – >2.20)	
	Age			
	6 – 12 months	23	1.88 (1.76 – >2.20)	0.015*
	13 – 24 months	62	>2.20 (1.93 – >2.20)	
	>24 months	95	>2.20 (1.87 – >2.20)	
	Breed			
	Brahman typical	16	2.10 (1.74 – >2.20)	0.580
	Brahman cross	79	>2.20 (1.87 – >2.20)	
	Nguni	85	>2.20 (1.88 – >2.20)	
	Location			
	Clare A	47	>2.20 (1.94 – >2.20)	0.115
	Eglington	43	1.97 (1.76 – >2.20)	
	Shorty	54	>2.20 (1.87 – >2.20)	
	Tlhavekisa	36	2.15 (1.87 – >2.20)	
56	**Sex**			
	Female	87	1.99 (1.78 – >2.20)	0.936
	Male	31	1.97 (1.78 – >2.20)	
	Age			
	6 – 12 months	16	2.14 (1.79 – >2.20)	0.891
	13 – 24 months	39	1.97 (1.81 – >2.20)	
	>24 months	63	1.99 (1.77 – >2.20)	
	Breed			
	Brahman typical	8	1.89 (1.68 – 2.22)	0.601
	Brahman cross	58	1.96 (1.78 – >2.20)	

	Nguni	52	2.17 (1.78 – >2.20)	
	Location			
	Eglington	36	1.96 (1.82 – >2.20)	0.031*
	Shorty	45	1.86 (1.71 – >2.20)	
	Tlhavekisa	37	>2.20 (1.94 – >2.20)	
70	**Sex**			
	Female	44	1.78 (1.67 – 1.98)	0.166
	Male	17	1.85 (1.75 – 2.14)	
	Age			
	6 – 12 months	5	1.67 (1.59 – 1.85)	0.293
	13 – 24 months	22	1.84 (1.76 – 2.22)	
	>24 months	34	1.83 (1.67 – 2.02)	
	Breed			
	Brahman typical	4	1.73 (1.69 – 1.79)	0.243
	Brahman cross	25	1.85 (1.75 – 2.12)	
	Nguni	35	1.81 (1.67 – 2.10)	
	Location			
	Clare A	10	1.84 (1.71 – 2.06)	0.505
	Eglington	17	1.82 (1.67 – 1.94)	
	Shorty	17	1.85 (1.77 – 2.18)	
	Tlhavekisa	17	1.75 (1.66 – 2.16)	
84	**Sex**			
	Female	23	1.99 (1.75 – >2.20)	0.158
	Male	8	1.82 (1.66 – 1.92)	

	Age			
	6 – 12 months	1	2.79	0.207
	13 – 24 months	12	1.82 (1.73 – 2.28)	
	>24 months	18	1.95 (1.73 – >2.20)	
	Breed			
	Brahman typical	6	1.89 (1.72 – 2.69)	0.902
	Brahman cross	9	1.75 (1.71 – >2.20)	
	Nguni	16	1.95 (1.77 – 2.28)	
	Location			
	Clare A	23	1.93 (1.76 – >2.20)	0.255
	Eglington	8	1.83 (1.64 – 2.22)	
98	**Sex**			
	Female	60	1.85 (1.67 – >2.20)	0.994
	Male	14	1.83 (1.74 – 1.90)	
	Age			
	6 – 12 months	7	1.86 (1.65 – >2.20)	0.464
	13 – 24 months	22	1.75 (1.71 – 2.02)	
	>24 months	45	1.89 (1.68 – >2.20)	
	Breed			
	Brahman typical	8	1.82 (1.69 – 2.21)	0.464
	Brahman cross	29	1.75 (1.65 – >2.20)	
	Nguni	37	1.85 (1.73 – >2.20)	
	Location			
	Clare A	18	1.81 (1.66 – 2.20)	0.609
	Eglington	6	1.89 (1.76 – 2.47)	

	Shorty	17	1.75 (1.67 – >2.20)	
	Tlhavekisa	33	1.85 (1.71 – >2.20)	
112	**Sex**			
	Female	108	1.93 (1.73 – 2.20)	0.002*
	Male	25	1.74 (1.67 – 1.92)	
	Age			
	6 – 12 months	12	1.75 (1.65 – 2.08)	0.202
	13 – 24 months	43	1.87 (1.72 – >2.20)	
	>24 months	78	1.92 (1.73 – >2.20)	
	Breed			
	Brahman typical	17	1.77 (1.70 – 1.92)	0.096
	Brahman cross	60	1.85 (1.72 – 2.26)	
	Nguni	56	1.95 (1.73 – >2.20)	
	Location			
	Clare A	27	1.77 (1.70 – 1.95)	0.058
	Eglington	34	1.77 (1.72 – 2.18)	
	Shorty	30	1.92 (1.67 – 2.03)	
	Tlhavekisa	42	2.06 (1.74 – >2.20)	

Table 11. Median and interquartile range (IQR) log_{10} antibody titre for SAT 2 by animal factors and location

Day	Variable	N	Median (IQR)	P-value
0	**Sex**			
	Female	28	1.87 (1.75 – >2.20)	0.705
	Male	5	1.84 (1.75 – 2.00)	
	Age			
	6 – 12 months	1	>2.20	0.477
	13 – 24 months	12	1.84 (1.69 – >2.20)	
	>24 months	20	1.85 (1.77 – 2.18)	
	Breed			
	Brahman typical	10	1.90 (1.82 – >2.20)	0.631
	Brahman cross	11	1.83 (1.73 – >2.20)	
	Nguni	12	1.87 (1.74 – 2.28)	
	Location			
	Clare A	21	1.84 (1.74 – >2.20)	0.678
	Eglington	12	1.89 (1.78 – 2.22)	
14	**Sex**			
	Female	147	>2.20 (>2.20 – >2.20)	0.535
	Male	52	>2.20 (>2.20 – >2.20)	
	Age			
	6 – 12 months	30	>2.20 (1.83 – >2.20)	0.021*
	13 – 24 months	63	>2.20 (>2.20 – >2.20)	
	>24 months	106	>2.20 (>2.20 – >2.20)	
	Breed			

	Brahman typical	20	>2.20 (>2.20 – >2.20)	0.392
	Brahman cross	92	>2.20 (>2.20 – >2.20)	
	Nguni	87	>2.20 (>2.20 – >2.20)	
	Location			
	Clare A	41	>2.20 (>2.20 – >2.20)	0.262
	Eglington	51	>2.20 (>2.20 – >2.20)	
	Shorty	60	2.20 (2.15 – >2.20)	
	Tlhavekisa	29	2.20 (>2.20 – >2.20)	
28	**Sex**			
	Female	101	1.88 (1.77 – >2.20)	0.355
	Male	36	1.84 (1.74 – >2.20)	
	Age			
	6 – 12 months	17	1.83 (1.67 – >2.20)	0.279
	13 – 24 months	47	>2.20 (1.82 – >2.20)	
	>24 months	73	1.86 (1.76 – >2.20)	
	Breed			
	Brahman typical	18	2.10 (1.76 – >2.20)	0.623
	Brahman cross	67	1.86 (1.75 – >2.20)	
	Nguni	52	1.88 (1.77 – >2.20)	
	Location			
	Clare A	20	2.10 (1.73 – >2.20)	0.638
	Eglington	31	1.88 (1.76 – >2.20)	
	Shorty	44	2.15 (1.78 – >2.20)	
	Tlhavekisa	42	1.84 (1.76 – >2.20)	
42	**Sex**			

	Female	158	>2.20 (>2.20 – >2.20)	0.445
	Male	58	>2.20 (>2.20 – >2.20)	
	Age			
	6 – 12 months	33	>2.20 (2.12 – >2.20)	0.025*
	13 – 24 months	69	>2.20 (>2.20 – >2.20)	
	>24 months	114	>2.20 (>2.20 – >2.20)	
	Breed			
	Brahman typical	20	>2.20 (>2.20 – >2.20)	0.606
	Brahman cross	101	>2.20 (>2.20 – >2.20)	
	Nguni	95	>2.20 (>2.20 – >2.20)	
	Location			
	Clare A	51	>2.20 (>2.20 – >2.20)	0.054
	Eglington	50	>2.20 (>2.20 – >2.20)	
	Shorty	61	>2.20 (>2.20 – >2.20)	
	Tlhavekisa	54	>2.20 (>2.20 – >2.20)	
56	**Sex**			
	Female	77	2.15 (1.76 – >2.20)	0.041*
	Male	28	2.08 (1.86 – >2.20)	
	Age			
	6 – 12 months	16	1.99 (1.75 – >2.20)	0.684
	13 – 24 months	29	2.15 (1.79 – >2.20)	
	>24 months	60	1.97 (1.74 – >2.20)	
	Breed			
	Brahman typical	8	2.12 (1.96 – >2.20)	0.720
	Brahman cross	47	1.96 (1.75 – >2.20)	

	Nguni	50	1.99 (1.74 – >2.20)	
	Location			
	Eglington	32	>2.20 (1.94 – >2.20)	0.132
	Shorty	38	1.88 (1.72 – >2.20)	
	Tlhavekisa	35	1.92 (1.77 – >2.20)	
70	**Sex**			
	Female	61	1.81 (1.71 – 1.89)	0.391
	Male	26	1.75 (1.66 – 1.87)	
	Age			
	6 – 12 months	6	1.78 (1.74 – 1.96)	0.258
	13 – 24 months	30	1.82 (1.73 – >2.20)	
	>24 months	51	1.76 (1.65 – 1.86)	
	Breed			
	Brahman typical	8	1.79 (1.68 – 2.18)	0.291
	Brahman cross	37	1.75 (1.65 – 1.86)	
	Nguni	42	1.82 (1.72 – >2.20)	
	Location			
	Clare A	14	1.82 (1.70 – >2.20)	0.133
	Eglington	20	1.82 (1.70 – 1.86)	
	Shorty	30	1.85 (1.70 – >2.20)	
	Tlhavekisa	23	1.74 (1.65 – 1.79)	
84	**Sex**			
	Female	21	1.93 (1.83 – >2.20)	0.616
	Male	6	1.93 (1.79 – 2.03)	

	Age			
	6 – 12 months	1	>2.20	0.158
	13 – 24 months	14	1.86 (1.82 – 1.94)	
	>24 months	12	>2.20 (1.82 – >2.20)	
	Breed			
	Brahman typical	5	1.93 (1.82 – 2.11)	0.977
	Brahman cross	10	1.86 (1.83 – >2.20)	
	Nguni	12	1.95 (1.82 – >2.20)	
	Location			
	Clare A	20	1.93 (1.82 – >2.20)	0.889
	Eglington	7	1.93 (1.82 – >2.20)	
98	**Sex**			
	Female	80	1.85 (1.75 – 2.89)	0.102
	Male	17	1.74 (1.67 – >2.20)	
	Age			
	6 – 12 months	8	1.76 (1.66 – 2.22)	0.353
	13 – 24 months	34	1.79 (1.67 – 2.03)	
	>24 months	55	1.89 (1.74 – 2.20)	
	Breed			
	Brahman typical	12	1.92 (1.81 – >2.20)	0.132
	Brahman cross	39	1.76 (1.68 – 1.94)	
	Nguni	46	1.87 (1.68 – >2.20)	
	Location			
	Clare A	23	1.87 (1.69 – >2.20)	0.484
	Eglington	15	1.85 (1.64 – 2.15)	

	Shorty	24	1.78 (1.67 – 1.99)	
	Tlhavekisa	35	1.87 (1.74 – >2.20)	
112	**Sex**			
	Female	121	1.95 (1.75 – >2.20)	0.004*
	Male	35	1.79 (1.69 – 1.99)	
	Age			
	6 – 12 months	20	1.80 (1.70 – >2.20)	0.384
	13 – 24 months	50	1.93 (1.75 – >2.20)	
	>24 months	86	1.95 (1.75 – >2.20)	
	Breed			
	Brahman typical	18	1.97 (1.92 – 2.20)	0.356
	Brahman cross	67	1.87 (1.75 – 2.20)	
	Nguni	71	1.94 (1.74 – >2.20)	
	Location			
	Clare A	32	1.95 (1.77 – >2.20)	0.708
	Eglington	37	1.91 (1.69 – >2.20)	
	Shorty	44	1.95 (1.75 – >2.20)	
	Tlhavekisa	43	1.89 (1.75 – >2.20)	

Table 12. Median and interquartile range (IQR) log_{10} antibody titre for SAT 3 by animal factors and location

Day	Variable	N	Median (IQR)	P-value
0	**Sex**			
	Female	12	1.76 (1.69 – 2.19)	0.223
	Male	1	>2.20	
	Age			
	6 – 12 months	1	>2.20	0.429
	13 – 24 months	2	1.75	
	>24 months	10	1.79 (1.67 – >2.20)	
	Breed			
	Brahman typical	2	>2.20	0.152
	Brahman cross	6	1.73 (1.65 – 1.95)	
	Nguni	5	1.76 (1.71 – 2.08)	
	Location			
	Clare A	7	1.76 (1.74 – >2.20)	0.277
	Eglington	6	1.75 (1.65 – 1.97)	
14	**Sex**			
	Female	131	>2.20 (1.95 – >2.20)	0.555
	Male	51	>2.20 (1.83 – >2.20)	
	Age			
	6 – 12 months	23	1.95 (1.75 – >2.20)	0.198
	13 – 24 months	58	>2.20 (1.96 – >2.20)	
	>24 months	101	>2.20 (1.93 – >2.20)	
	Breed			

	Brahman typical	18	>2.20 (1.89 – >2.20)	0.908
	Brahman cross	84	>2.20 (1.95 – >2.20)	
	Nguni	80	>2.20 (1.87 – >2.20)	
	Location			
	Clare A	46	>2.20 (1.86 – >2.20)	0.645
	Eglington	62	>2.20 (1.86 – >2.20)	
	Shorty	40	2.20 (1.96 – >2.20)	
	Tlhavekisa	29	2.20 (1.96 – >2.20)	
28	**Sex**			
	Female	96	>2.20 (1.86 – >2.20)	0.338
	Male	38	2.22 (1.88 – >2.20)	
	Age			
	6 – 12 months	14	>2.20 (1.93 – >2.20)	0.858
	13 – 24 months	46	>2.20 (1.86 – >2.20)	
	>24 months	74	>2.20 (1.86 – >2.20)	
	Breed			
	Brahman typical	16	>2.20 (1.94 – >2.20)	0.676
	Brahman cross	65	>2.20 (1.84 – >2.20)	
	Nguni	53	>2.20 (1.88 – >2.20)	
	Location			
	Clare A	24	>2.13 (1.76 – >2.20)	0.226
	Eglington	29	>2.20 (1.87 – >2.20)	
	Shorty	42	>2.20 (1.87 – >2.20)	
	Tlhavekisa	39	>2.20 (1.96 – >2.20)	
42	**Sex**			

	Female	150	>2.20 (>2.20 – >2.20)	0.011*
	Male	55	>2.20 (2.16 – >2.20)	
	Age			
	6 – 12 months	29	>2.20 (2.10 – >2.20)	0.002*
	13 – 24 months	66	>2.20 (>2.20 – >2.20)	
	>24 months	110	>2.20 (>2.20 – >2.20)	
	Breed			
	Brahman typical	19	>2.20 (1.96 – >2.20)	0.033*
	Brahman cross	97	>2.20 (>2.20 – >2.20)	
	Nguni	89	>2.20 (>2.20 – >2.20)	
	Location			
	Clare A	48	>2.20 (>2.20 – >2.20)	0.001*
	Eglington	44	>2.20 (1.78 – >2.20)	
	Shorty	59	>2.20 (>2.20 – >2.20)	
	Tlhavekisa	54	>2.20 (>2.20 – >2.20)	
56	**Sex**			
	Female	66	1.94 (1.78 – >2.20)	0.755
	Male	23	1.97 (1.78 – >2.20)	
	Age			
	6 – 12 months	16	2.10 (1.76 – >2.20)	0.720
	13 – 24 months	25	1.96 (1.86 – >2.20)	
	>24 months	48	1.94 (1.77 – 2.29)	
	Breed			
	Brahman typical	9	1.96 (1.80 – 2.22)	0.429
	Brahman cross	45	1.92 (1.77 – >2.20)	

	Nguni	35	1.99 (1.79 – >2.20)	
	Location			
	Eglington	26	1.92 (1.77 – >2.20)	0.225
	Shorty	31	1.91 (1.75 – >2.20)	
	Tlhavekisa	32	2.13 (1.88 – >2.20)	
70	**Sex**			
	Female	57	1.83 (1.75 – 2.15)	0.158
	Male	20	1.96 (1.76 – >2.20)	
	Age			
	6 – 12 months	7	1.83 (1.77 – 2.18)	0.984
	13 – 24 months	25	1.89 (1.74 – >2.20)	
	>24 months	45	1.85 (1.77 – 2.25)	
	Breed			
	Brahman typical	7	1.78 (1.65 – 1.93)	0.259
	Brahman cross	36	1.82 (1.73 – 2.27)	
	Nguni	34	1.94 (1.79 – >2.20)	
	Location			
	Clare A	10	>2.20 (1.79 – >2.20)	0.084
	Eglington	20	1.77 (1.72 – 1.94)	
	Shorty	24	1.85 (1.74 – >2.20)	
	Tlhavekisa	23	1.89 (1.78 – >2.20)	
84	**Sex**			
	Female	19	1.88 (1.72 – >2.20)	0.749
	Male	8	1.83 (1.78 – 2.13)	

Age			
6 – 12 months	2	2.04	0.250
13 – 24 months	12	1.77 (1.72 – 1.98)	
>24 months	13	1.88 (1.81 – >2.20)	
Breed			
Brahman typical	5	1.78 (1.74 – 2.11)	0.721
Brahman cross	10	1.90 (1.75 – >2.20)	
Nguni	12	1.84 (1.71 – >2.20)	
Location			
Clare A	17	1.88 (1.75 – >2.20)	0.687
Eglington	10	1.83 (1.74 – >2.20)	
Sex			
Female	70	1.98 (1.78 – >2.20)	0.116
Male	24	1.83 (1.72 – 2.21)	
Age			
6 – 12 months	10	2.10 (1.71 – 2.36)	0.884
13 – 24 months	32	1.91 (1.76 – 2.27)	
>24 months	52	1.95 (1.75 – >2.20)	
Breed			
Brahman typical	12	2.10 (1.83 – >2.20)	0.133
Brahman cross	43	1.83 (1.71 – >2.20)	
Nguni	39	1.97 (1.81 – >2.20)	
Location			
Clare A	20	1.93 (1.81 – >2.20)	0.506
Eglington	12	1.86 (1.74 – >2.20)	

98

	Shorty	22	1.83 (1.72 – >2.20)	
	Tlhavekisa	40	1.98 (1.81 – 2.88)	
112	**Sex**			
	Female	69	1.88 (1.73 – >2.20)	0.265
	Male	9	1.79 (1.63 – 2.14)	
	Age			
	6 – 12 months	4	2.11 (1.77 – 2.35)	0.300
	13 – 24 months	26	1.78 (1.65 – 2.10)	
	>24 months	48	1.91 (1.73 – >2.20)	
	Breed			
	Brahman typical	9	1.79 (1.68 – 2.11)	0.150
	Brahman cross	35	1.84 (1.65 – 1.97)	
	Nguni	34	1.94 (1.75 – >2.20)	
	Location			
	Clare A	16	1.82 (1.67 – >2.20)	0.801
	Eglington	18	1.83 (1.71 – >2.20)	
	Shorty	15	1.84 (1.66 – 2.00)	
	Tlhavekisa	29	1.93 (1.74 – >2.20)	

Table 13. Estimated marginal means for rounds, sex, age and FMD serotypes based on the mixed effect linear model that included random effects for dip tank and herd

Variable	Mean Log_{10} titre*	95%CI	P-value
Day			<0.001*
0	1.34[a]	1.27 - 1.40	
14	1.97[b]	1.92 - 2.02	
28	1.71[b]	1.66 - 1.76	
42	2.06[b]	2.01 - 2.12	
56	1.65[b]	1.59 - 1.70	
70	1.45[b]	1.40 - 1.51	
84	1.53[b]	1.47 - 1.59	
98	1.55[b]	1.49 - 1.60	
112	1.57[b]	1.52 - 1.62	
Sex			0.018*
Male	1.62	1.56 - 1.68	
Female	1.68	1.63 - 1.73	
Age category			<0.001*
6-12 months	1.56[a]	1.49 - 1.63	
13-24 months	1.69[b]	1.63 - 1.75	
>24 months	1.70[b]	1.65 - 1.75	
Serotypes			<0.001*
SAT 1	1.61[a]	1.56 - 1.66	

SAT 2	1.71[b]	1.66 - 1.75
SAT 3	1.63[a]	1.58 - 1.68

CI = confidence interval

*Means without superscripts in common at statistically different after Bonferroni correction of P values

Table 14. Estimates of marginal means for FMD serotypes based on the mixed effect linear model that included random effects for dip tank and herd

Serotype	Day	Mean Log_{10} titre	95%CI
SAT 1	0	1.20	1.12 – 1.28
	14	1.91	1.85 – 1.97
	28	1.70	1.64 – 1.76
	42	1.89	1.84 – 1.96
	56	1.69	1.64 – 1.76
	70	1.40	1.34 – 1.46
	84	1.59	1.51 – 1.67
	98	1.48	1.42 – 1.54
	112	1.59	1.54 – 1.66
SAT 2	0	1.49	1.42 – 1.58
	14	2.06	2.00 – 2.12
	28	1.69	1.64 – 1.76
	42	2.19	2.13 – 2.25
	56	1.65	1.59 – 1.71
	70	1.48	1.42 – 1.54
	84	1.52	1.44 – 1.59
	98	1.57	1.51 – 1.63
	112	1.69	1.63 – 1.74
SAT 3	0	1.32	1.23 – 1.39
	14	1.93	1.87 – 1.99
	28	1.74	1.68 – 1.79
	42	2.11	2.10 – 2.17

56	1.59	1.54 – 1.66
70	1.47	1.41 – 1.53
84	1.49	1.40 – 1.57
98	1.60	1.54 – 1.66
112	1.43	1.37 – 1.49

CI = confidence interval.

4.4 Discussion

The proportion of cattle with meaningful titres to previous vaccination was low suggesting that the interval between vaccinations was too long to maintain adequate vaccinal titres in sampled cattle. However, by 14 days post vaccination a large proportion of the study population had seroconverted to all SAT serotypes indicating a serological response to vaccination. This is consistent with a previous study in which vaccinated cattle produced significant antibody titres between 14 and 28 days after a single vaccination (Doel, 2003). In a related study involving dairy herds in Saudi Arabia, it has also been demonstrated that maximum antibody titres are typically reached 7-10 days post-vaccination with a vaccine containing serotype A antigen (Woolhouse, et al., 1996).

Duration of immunity is an important consideration for FMD vaccines (Hunter, 1998; Doel, 2003). A vaccine that will induce a strong serological response with a sustained duration is required for effective control (Cloete, et al., 2008). However, aqueous FMD vaccines are often unable to provide a sustained immunity in ruminants exceeding 4-6 months. Thus, cattle in endemic areas require revaccination at regular intervals of 4-6 months to ensure protective levels of antibodies (Cox, et al., 2003). In this study, vaccination did not elicit sustained immune response beyond 4 months for the majority of enrolled cattle. In a previous study in Saudi Arabia where FMD outbreaks persist in dairy cattle herds, despite

revaccination at intervals of 4-6 months, it has been observed that the critical inter-vaccination intervals which would provide herd immunity against FMDV is unrealistically short, especially for heterologous challenge (Woolhouse, et al., 1996).

SAT 2 antibody responses were observed to be more consistent relative to SAT 1 and SAT 3 viruses. This is however, consistent with the findings in which a bivalent FMD vaccine demonstrated strong antibody response to SAT 2 in vaccinated cattle (Massicame, 2012).

Age was a significant predictor of titre with adult cattle (>24 months of age) having higher antibody titres compared to younger cattle. This might be due to repeated exposures to the vaccine and subsequent anamnestic response. Weanling cattle (6-12 months) had lower titres, which might have been caused by the interference of maternally derived antibodies since it is a common practice to vaccinate very young calves during mass vaccination campaigns.

Female cattle had higher titres compared to male cattle. There is no biological explanation as to why female cattle would mount a better serological response since vaccination occurs at the same time and all animals are managed within the same system. A possible explanation is that female cattle might be presented for vaccination more regularly compared to male cattle due to the difficulty in handling and restraint during vaccination campaigns. The indigenous local Nguni breed formed the majority of the cattle population within Mnisi communal area implying a traditional livestock production system. However, breed did not have a significant effect on the serological responses of enrolled cattle.

Location was not a significant predictor of titre when evaluated independently per sampling time and serotype (with an occasional exception) suggesting that there was not an important effect of veterinary technician and therefore important differences in vaccine administration. However, an overall effect of the veterinary technician could not be tested since dip tank was included in the model as a random, rather than fixed effect.

This study is limited by the fact that it was based on serological responses only. Also the use of heterologous antigens in the liquid-phase blocking ELISA assay used would have underestimated the proportion of cattle classified as seropositive. The use of liquid phase blocking ELISA alone without virus neutralisation test is another limitation to this study. For Shorty and Tlhavekisa, animals were not sampled during the first week as a result of the on-going vaccination programme which could not permit simultaneous sampling of cattle at the dip tanks. Also by 48 days of the study farmers could not present their cattle for sampling at Shorty and Tlhavekisa as a result of excessive rainfall within the period. The selected cattle were not always available for complete follow-up as a result of some cattle missing during sampling at the dip tanks. The convenience sampling approach employed in this study might also be a potential cause of selection bias.

Overall, this study has demonstrated that high proportions of vaccinated cattle seroconvert within a period of 14 days post-vaccination, however the duration of immunity is unrealistically short considering the practice of 4-6 month inter-vaccination intervals as a common practice. It has however been recommended by the vaccine manufacturer that young calves should be revaccinated within one month after primary vaccination, but this is not always applicable in field situations and might be one of the reasons for this observation. Therefore, it will be necessary to undertake a wider study to identify the potential causes of the limited duration of immunity observed using the current vaccine. The vaccine should be applied as recommended by the manufacturer by revaccinating young calves. We recommend that this should be included as routine when farmers present animals for inspection at the dip tanks in addition to the annual mass vaccination campaign practiced in the study area.

5 SUMMARY AND CONCLUSIONS

The objective of these studies was to investigate the epidemiology and control of FMD at the wildlife/livestock interface of the Mnisi communal areas of Bushbuckridge, Mpumalanga South Africa. The studies were implemented in effort to understand the sociological aspects of FMD control and field performance of FMD vaccines routinely applied within the study area. No information was available concerning the perceptions of communal farmers related to FMD control prior to these studies.

Vaccination remains a fundamental tool for the control of FMD in the endemic countries of Africa. In most parts of the continent, FMD control is not a high priority and the disease is often under-reported. South Africa has a control policy for FMD that divides the country into disease free zone, control and infected zones. Cattle in the FMD control zone (with vaccination) are routinely vaccinated against FMD using inactivated trivalent (SAT 1-3) alhydrogel-saponin preparations. However, information was not available concerning the effectiveness of this programme for sustaining an immune response adequate to prevent disease when exposed to field strains of the FMD virus.

To understand the perceptions of communal farmers concerning FMD control at the interface area, a structured questionnaire was administered to farmers at the 15 community dip tanks within the study area. One hundred and four farmers participated in the questionnaire interview with the majority of respondents indicating high levels of satisfaction with the current disease control programmes. However, the more educated farmers indicated a lower level of satisfaction with the programmes and non-satisfied respondents were more likely to treat sick animals themselves rather than obtain professional veterinary services. The majority of respondents indicated that the African buffalo is a risk factor for FMD outbreaks at the interface.

A cross-sectional sampling of 286 cattle (within six months of vaccination) was performed to determine the proportion of cattle with presumed protective titres for FMD and the proportion of herds with adequate herd immunity. Relative to an antibody titre of ≥1.6 $Log_{10,}$ the seroprevalence was 20%, 39% and 22% to SAT 1-3, respectively over a median period of 189 days since most recent vaccination. Median titres for each serotype varied among herds and dip tanks. However, seropositivity was less than 80% for all SAT serotypes in all but a single herd. Antibody responses to SAT 2 were more pronounced relative to SAT 1 and SAT 3.

Two hundred and ninety-three cattle from 4 community dip tanks in the study area were selected and longitudinally followed for 112 days following the routine mass vaccination campaign implemented by the provincial veterinary services. Seroprevalence to previous vaccination in cattle sampled at the start of the study (Day 0) varied from 3 – 47% for the SAT virus types. The proportion of seropositive cattle increased for all SAT virus types at 14 days post-vaccination with the highest proportions for SAT 2. Antibody responses peaked up in the range of 65% - 98%, at 42 days post-vaccination for all the SAT type viruses until starting to decline at 56 days post-vaccination. By the end of the study period, antibody responses to all SAT virus serotypes was less than 30% at each of the study locations for a titre threshold of ≥2 log_{10}. Virus serotype was observed to be a predictor of antibody titre with SAT 2 having higher titres and the duration of the serological response varied by serotype. Female cattle had higher titres than male. Vaccinal titres also varied by age with older cattle tending to have better serological responses.

Understanding the perceptions of livestock owners concerning a disease control intervention is critical because perceptions affect the decision to adopt a new technology or innovation. There has been an increase in the occurrence of FMD outbreaks despite the sustained efforts in routine prophylactic vaccination of cattle at the interface, with the Mnisi area having recent

recurring outbreaks for a period of over 8 months. There is a need to develop a cost effective vaccination programme that will induce effective herd immunity in effort to reduce the number and extent of outbreaks. Post-vaccination monitoring of herd immunity should form an integral part of all FMD vaccination programmes within southern Africa. Findings from this study will be useful in planning and implementing the progressive control of FMD at the wildlife/livestock interface of southern Africa by reviewing the current vaccination regimen and applying vaccines with appropriate topotypes.

Educated farmers should be consulted during the design of progressive FMD control programmes within communal areas. The involvement of educated farmers will improve acceptance of control options and overall programme implementation. The current FMD vaccination programme should be critically evaluated since results suggest an inadequate level of herd immunity. The field application of FMD vaccines including vaccine storage and handling, maintenance of the cold chain during transportation, administration dosage and routes of application should be studied to identify potential explanations for the limited duration of vaccinal titres and poor serological responses.

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APPENDIX I

QUESTIONNAIRE

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

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

QUESTIONNAIRE FOR THE EVALUATION OF HERD LEVEL FMD VACCINATION COVERAGE WITHIN THE MNISI COMMUNITY SOUTH AFRICA

The University of Pretoria, Faculty of Veterinary Science is conducting a study on herd-level FMD vaccination coverage of cattle within the Mnisi (Bushbuckridge) study region, Mpumalanga, South Africa.

You have been selected as one of our respondent 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)..... □ Owner □ Handler
2. Address.....
3. Gender □ M □ F
4. Date of birth.....
5. Marital status □ Single □ Married □ Divorced □ Widow

6. The highest level of education that you have completed.....
7. What is your most important source of income?

   □ Livestock □ Crop □ Other (specify).....

8. Other occupation.....

SECTION B (HERD MANAGEMENT)

1. How many cattle do you have today? .....
2. How many herds do you keep? .....
3. Which of the following animals do you keep in addition to cattle?

   □ Pig □ Sheep □ Goat □ Buffalo (domestic) □ Kudu □ Chicken

4. When was the last time that you purchased animals? □ N/A Date.....
5. When was the last time you sold animals from your herd? □ N/A Date.....
6. When was the last time you introduced a new stock to your herd? □ N/A Date.....
7. When did you start raising animals? Year.....
8. What is the source of water to your animals during grazing? □ Pipe □ Well □ Pond

SECTION C (GENERAL DISEASE CONTROL)

1. Vaccination can reduce disease in animals.

   □ Yes □ No □ Unsure

2. Vaccination can make animals sick.

   □ Yes □ No □ Unsure

3. Vaccination can cause abortion in animals.

   □ Yes □ No □ Unsure

4. Vaccination improves the wellbeing of animals.

   □ Yes □ No □ Unsure

5. Vaccination can decrease feed consumption in animals.

   □ Yes □ No □ Unsure

6. Do you change the management of your animals after vaccination?

   □ Yes □ No

7. If yes, how.....

8. Should sick animals be presented for vaccination? □ Yes □ No

9. Should pregnant animals be presented for vaccination? □ Yes □ No

10. How satisfied are you with the dip tank vaccination programme?

    □ Very satisfied □ Little satisfied □ Not satisfied at all

11. How satisfied are you with the dip tank community?

    □ Very satisfied □ Little satisfied □ Not satisfied at all

12. Does the weekly dipping exercise reduce tick-borne disease? □ Yes □ No □ Unsure

13. Regular dipping of cattle waste time for grazing. □ Yes □ No □ Unsure

14. During dip tank session, much time is spent organising animals. □ Yes □ No

15. During dip tank session, less time is spent inspecting animals. □ Yes □ No

16. Which of the following is your favourite dip tank exercise?

    □ Inspection □ Dipping □ Vaccination Others (Specify).....

17. How frequently have your cattle been vaccinated? .....times

SECTION C (KNOWLEDGE OF FMD EPIDEMIOLOGY)

1. Do you know a disease that causes ulcers on the tongue, feet and udder of cattle?

   □ Yes □ No

2. What name do you call this disease in your native dialect? .....

3. When did you last observed this disease in your herd? □ Never Date.....

4. Have any of your animals died from this disease?

□ Yes □ No

5. What age groups were mostly affected? □ Young □ Adults
6. How do you manage disease animals?

   □ Call a vet. □Treat yourself □ Culling/sell □ Slaughter □ Euthanasia
7. Have you ever come in contact with wildlife during grazing? □ Yes □ No
8. Do you graze close to the park fence during typical periods of the season?

   □ Yes □ No
9. On the average how many kilometres do you graze a day? .....
10. Which of the following do you feel is responsible for this disease outbreak around the KNPI?

    □ Buffalo escape □ Impala escape □ grazing around park □ Introduction of new stock Other (specify).....

APPENDIX II

MANUSCRIPT I

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

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Abstract

Communal areas surrounding the Kruger National Park (KNP) are part of the Foot-and-mouth disease (FMD) protection zone with vaccination. Foot-and-mouth disease and its control affect the productivity of resource-poor farmers who depend on livestock for livelihood.

The objective of the present study was to evaluate the perceptions of farmers concerning FMD control and estimate the proportion of cattle with presumed protective titres for FMD and herds with adequate herd immunity at the wildlife/livestock interface within Mpumalanga Province, South Africa.

One hundred and four farmers responded to the questionnaire with 73% (76/104) being cattle owners while the remainder being hired cattle handlers. The majority of respondents, (79%, 95%CI: 70%-80%) indicated a high level of satisfaction with the current animal health programmes at the dip tanks. The educational level of respondents varied by satisfaction level with the median education level being standard 9 (IQR: 2-12) for non-satisfied respondents, standard 3 (IQR: 0-6) for little satisfied and standard 7 (IQR: 2-11) for very satisfied respondents (P = 0.036). Non-satisfied respondents were more likely to treat sick animals themselves rather than seek veterinary assistance (P = 0.002). The majority of respondents identified the African buffalo as a risk factor for FMD outbreaks (92%, 95%CI: 85%-96%). Relative to an antibody titre of $\geq$1.6 Log_{10} (1:40 dilution), 20% (95%CI: 14%-26%) of sampled cattle had serological evidence of vaccination to SAT 1, 39% (95%CI: 32%-46%) to SAT 2 and 22% (95%CI: 17%-27%) to SAT 3.

Sampled cattle had inadequate immune responses to the current FMD vaccination programme within the study area. The high satisfaction level expressed by the majority of respondents is

likely misguided and such factors should be considered when designing progressive control programmes at the wildlife/livestock interface.

Keywords: Foot-and-mouth-disease, vaccination, control, wildlife interface

1. Introduction

Foot-and-mouth disease (FMD) is one of the most economically important diseases of livestock in the tropics (Tanya, et al., 2003) and the disease is considered endemic in much of sub-Saharan Africa (Vosloo, et al., 2002b; Jori, et al., 2009). In South Africa, FMD is endemic in the Kruger National Park (KNP) due to the presence of African buffalo (*Syncerus caffer*) and the surrounding communities are classified as FMD protection zones with vaccination (DAFF, 2012). All three South African Territories serotypes (SAT 1, SAT 2 and SAT 3) of the FMD virus have been identified in African buffalo in the KNP and adjacent nature reserves (Vosloo, et al., 1995; Vosloo, et al., 2002b; Thomson, et al., 2003). African buffaloes can be FMDV carriers and have been associated with outbreaks in impala (*Aepycerus melampus*) within KNP and in cattle within the bordering communal areas (Thomson and Bastos, 2004; Vosloo, et al., 2009).

Resource-poor farmers frequently employ communal livestock production systems at interfaces with protected wildlife areas (Osofsky, 2005), where production outputs are often low because of husbandry practices, pasture quality and transmission of infectious diseases (Caron, et al., 2013).

Contacts among people, livestock and wildlife often occur at interface areas with the potential risk for disease transmission, which is a concern for communal farmers (de Garine-Wichatitsky, et al., 2012). Communal farmers raise livestock to produce milk, meat hides and manure that can be used to fertilise crop production (Barrett, 1992; Chimonyo, et al, 1999; Dovie, et al., 2006). Cattle also provide draught power for the cultivation of crops and transport of goods and services (Bayer, et al., 2004; Shackleton, et al., 2005). Livestock have been described as “inflation free banking” for resource-poor people and can be sold to pay for school fees, medical bills, village taxes and other household expenses (Dovie, et al., 2006).

Livestock farming reduces household food insecurity and poverty in communal areas (ISRDS, 2004; Coetzee, et al., 2006).

Disease control at the interface often employs vaccination and must consider issues related to delivery (LID, 1998; Heffernan and Misturelli, 2000) and characteristics of the adopter including perceptions and awareness of the technology in question (Bhattacharyya, et al., 1997; Bolorunduro, et al., 2004; Fandamu, et al., 2006; Homewood, et al., 2006). Important aspects to the adoption of animal health interventions among the poor are access, affordability and acceptability (Heffernan and Misturelli, 2000). The goal of vaccination campaigns is a wide-scale adoption at the community, national and even global levels (Mason and McGinnis, 1990; Humair, et al., 2002) and therefore programmes must consider the perceptions and feelings of the resource-poor farmers (McLeod and Rushton, 2007; Heffernan, et al., 2008).

Cattle at the interface with KNP are routinely vaccinated using a trivalent product containing all three SAT serotypes of FMDV. It has been recommended that at least 75% of the cattle population should be immunised (vaccinated and developed sufficient neutralising antibodies) to achieve herd immunity and prevent FMD virus transmission (Barteling, et al., 2004). Chemically inactivated FMD vaccines induce a short-lived duration of immunity similar to other inactivated vaccines (Hunter, 1998). Therefore, vaccine manufacturers typically recommend that cattle in an endemic setting are revaccinated after an initial double primary course at least three times a year (Woolhouse, et al., 1996).

The objective of the present study was to evaluate the perceptions of farmers concerning FMD control and estimate the proportion of cattle with presumed protective titres for FMD and herds with adequate herd immunity at the wildlife/livestock interface within Mpumalanga Province, South Africa.

2. Materials and methods

2.1 Research ethics

Ethical clearance was obtained at the University of Pretoria from the Animal Ethics Committee (Project Number V010-12) of the Faculty of Veterinary Science and the Research Ethics Committee (Project Number 2012-04-04) at the Faculty of Humanities. Section 20 approval (Animal Disease Act) was obtained from the Department of Agriculture, Forestry & Fisheries: Directorate of Animal Health (Application Number 12/11/1/1).

2.2 Study location and population

This study was conducted in 15 dip tanks of the Mnisi Community, Bushbuckridge Municipal Area Mpumalanga Province, South Africa. The Mnisi Community has a land area of 30,000 ha and a human population of 40,060 people living within 8,555 households. Domestic livestock include 14,400 heads of cattle owned by 1,300 farmers, 6,190 goats owned by 920 farmers and 330 pigs owned by 36 farmers (Statistics SA, 2001). Local household livelihoods are supplemented by land-based activities including cultivating home gardens, rearing livestock and gathering natural resources (Cousins, 1999; Shackleton, 2000; Dovie, et al., 2002).

The Bushbuckridge area has generally sandy and infertile granite soils. Rainfall occurs mainly during summer months (October-April) and the total amount varies from 800 mm per annum in the west to 500 mm per annum in the east. The increasing aridity moving eastward is accompanied by increasing variability in the mean annual rainfall and drought is a common occurrence in the district (Shackleton, 2000).

The main agricultural activity in the area is livestock farming with cattle as the most important species. Goats and chickens are locally abundant and there are also a few donkey and pig farmers. Two thirds of the land area forms an interface with the Kruger National Park

and provincial and private game reserves. Cattle and wildlife are separated by game-proof fences and the entire study region is situated within the FMD protection zone with vaccination. Cattle are routinely vaccinated using a trivalent vaccine (SAT 1, SAT 2 & SAT 3) and weekly clinical surveillance is conducted.

2.3 Study design and sample size justification

Cross-sectional studies using a structured questionnaire through in-person interview and a cluster sampling of cattle by herds were implemented. Sampling was conducted during May to June 2012 I the 15 community dip tanks of the Mnisi area. The sample sizes were calculated to estimate the proportion of respondents with knowledge concerning FMD epidemiology and control and the proportion of cattle with presumed titres to vaccination. For the cross-sectional interview, a percentage of 50% was assumed since there was no prior information and it was desired to estimate this proportion with 10% absolute error at the 95% level of confidence (Open Epi, version 2.3.1, Open Source Epidemiological Statistics for Public Health calculator – SS propor software). The sample size was estimated as 97 but was increased to 104 to sample 10% of farmers registered at each dip tank.

The sample size was calculated to estimate the expected herd-level seropositivity (herd with ≥80% seropositive animals) with a 20% absolute error and at the 95% level of confidence. The calculated sample size was 24 herds, however 30 herds were selected to allow for the enrolment of two herds per dip tank. Two herds were selected at each dip tank using a list of farmers from animal health technicians and within each herd ten cattle (or the entire herd when <10) were selected. All cattle at least 6 months of age (eligible for vaccination at the previous vaccination session) were sampled.

2.4 Questionnaire development and administration

A semi-structured questionnaire was designed to evaluate perceptions of communal farmers concerning FMD epidemiology and control at the wildlife/livestock interface. The questionnaire included multiple choice, dichotomous (yes/no), ordinal scale and free numerical or text responses focusing on the respondent's level of education and experience. Questions addressed owner demographics, herd management practices, general disease control and knowledge of FMD epidemiology.

Collected socio-demographic data included age, gender, marital status, education level, and sources of household income. Herd management data included the number of livestock, amount of time since the most recent purchase/sale of animals, duration of livestock farming and source of livestock drinking water. General disease control data included knowledge of FMD vaccination, satisfaction with the routine vaccination programme, satisfaction with dipping, favourite dip tank activities and annual frequency of FMD vaccination.

Data were collected concerning knowledge of the clinical signs of FMD, history of previous FMD outbreaks, disease management, and perceived risk factors for FMD outbreaks.

A composite vaccination score was created concerning factors that might affect farmers' participation in a vaccination campaign. This score was a summation with favourable responses assigned +1, unfavourable responses -1 and uncertain responses 0 marks. Questions included: vaccination can reduce disease in cattle, vaccination can make cattle sick, vaccination can cause abortion in cattle, vaccination improves cattle wellbeing, vaccination can reduce feed intake in cattle, sick cattle should be presented for vaccination and pregnant cattle should be presented for vaccination.

Questionnaires were administered through an in-person interview in the local language (Shangaan) after translation from English. Within each community dip tank, 10% of the

registered livestock owners/handlers were conveniently selected as they presented their cattle for inspection. The study was conducted in May and June 2012, a period that coincided with the routine FMD mass vaccination campaign in the area.

Farmers were eligible for enrolment if they attended a dip tank session on the day of the interview and those who regularly accompany their cattle for grazing. Participation was voluntary and a unique questionnaire identification number was used to maintain participant confidentiality. FMD vaccination history was extracted from owner-stock card and animal health technician livestock registers at the time of questionnaire administration. Official veterinary reports were retrospectively reviewed to confirm data concerning FMD vaccination.

2.5 Cattle sampling and testing

Farmers were conveniently selected as they presented their cattle for FMD inspection and dipping for ectoparasites. Eligible cattle were selected based on their order of presentation. Whole blood samples were collected from the mid-coccygeal or jugular vein into 10 ml vacutainer® tubes using Precision glide® needles (Becton, Dickinson and company, Franklin Lakes, New Jersey, USA). Blood was allowed to clot at ambient temperature in the field and transported to the laboratory within 6 hours of collection. Blood samples were centrifuged in the laboratory at 1450 g for 10 minutes. Serum was decanted into sterile cryovials and stored at -20°C until testing. Sera were packaged according to the Regulations of the National Road Traffic Act, 1996 (Act No. 93 of 1996) of the Republic of South Africa and transported on ice to the Transboundary Animal Diseases Programme Laboratory of the Onderstepoort Veterinary Institute (TADP), Pretoria for testing. Serum samples were tested for antibodies against FMDV structural proteins using liquid-phase blocking ELISA (Hamblin, et al., 1986b) employing TADP developed reagents for SAT 1, SAT 2 and SAT 3. Cattle were categorised as seropositive if the ELISA titre was 1.6 $\log_{10}$ or greater for each serotype.

2.6 Data analysis

Categorical data were described with percentages and 95% confidence intervals (CI) and continuous data were described using medians and interquartile ranges (IQR). Chi-square and Fisher's exact tests were used to compare proportions across categorical variables and Kruskal-Wallis (K-W) tests were used to compare factors for quantitative (non-normal) data. Significance was set as $P<0.05$. Descriptive data analysis was performed with EpInfoTM (Centre for Disease Control and Prevention, Atlanta, GA, USA), Open Epi (Open Source Epidemiological Statistics for Public Health), version 2.3.1, www.OpenEpi.com and Minitab (version16 State College, PA, USA). Commercially available software (IBM SPSS Statistics Version 21, International Business Machines Corp., Armonk, New York, USA) was used to estimate the seroprevalence while adjusting for clustered sampling and the different population size of cattle at each dip tank.

3. Results

3.1 Questionnaire results

One hundred and four respondents participated. The majority of respondents (73%; 76/104) were owners while the remainder were hired cattle handlers. Eighty-four percent of respondents (87/104) were male. Twenty-six percent (27/104) were single, 55% (57/104) married, 4% (4/104) divorced and 15% (15/104) widowed. The median age of respondents was 48 (interquartile range: 33-66) years. Twenty-one percent (22/104) of the respondents had no formal education, 38% (40/104) had completed primary education, 36% (37/104) completed secondary education and 1 (1/104) respondent had completed tertiary education.

The median (IQR) number of cattle owned by respondents was 11 (6-19) cattle. The median (IQR) time since the most recent purchase of cattle in the herd was 4 (2-13) years, the median time since the last sale was 1 (1-2) years, the median time since last introduction was 2 years

(1.5-2), and the median time in livestock farming was 13.5 (6-73) years. Married households had a median herd size of 8 (interquartile range: 5-15) versus 9.5 (interquartile range: 6-15) for the other categories combined (P = 0.418). The median (IQR) frequency of FMD vaccination reported by respondents was 2 (2-3) times per year. Reported herd vaccination from the owner-stock cards was in complete concordance with animal health technicians' register and the provincial veterinary service data base.

All respondents indicated livestock farming as their major source of income and 11% (11/104) indicated crop farming in addition to livestock. Other raised animals included: 14% (14/104) of respondents reared pigs, 39% (40/104) of respondents reared goats and 74% (77/104) of respondents reared chickens. Three respondents (3/104) indicated using pipe water as a source of livestock drinking water, 82% (85/104) indicated using well water and 18% (19/104) indicated the use of ponds.

Eighty-eight percent (92/104) of respondents indicated that out of all activities undertaken at the dip tank, dipping against ticks and ectoparasites was the favourite. Ninety-six percent of respondents (100/104) reported that they called a veterinarian whenever there is a problem in their herds, while 14% (15/104) indicated self-treatment as an option in addition to contacting a veterinarian.

The highest perceived risk for FMD outbreaks among respondent's cattle was buffalo escape from the park (92%; 96/104), followed by the introduction of new animals to the herds (9%; 9/104) and grazing adjacent to park fences (7%; 7/104). Twelve percent (12/104) of respondents reported that they knew of a disease that can cause lesions on the tongue, feet and udder. Nineteen percent (20/104) of respondents reported contacts with wildlife during grazing. Average daily grazing distance was variable with the majority of respondents (91%; 95/104) reporting a daily average of 1-10km.

Seventy-nine percent (82/104) of respondents were very satisfied with the current vaccination programme, 16% (17/104) were little satisfied and 5% (5/104) were not satisfied at all. Disease management strategies and crop production varied based on the satisfaction level (Table 1). The median education level of respondents varied over levels of satisfaction (P = 0.036) and was standard 9 (interquartile range: 2-12) for non-satisfied respondents, standard 3 (interquartile range: 0-6) for the little satisfied respondents and standard 7 (interquartile range: 2-11) for very satisfied respondents (Table 2). Non-satisfied respondents were more likely to treat sick animals themselves rather than seek veterinary assistance (P = 0.002).

The majority of respondents had favourable perceptions to vaccination (Table 3), however, 64% (67/103) believed that pregnant animals should not be presented for vaccination. The vaccination perception score of respondents varied over level of satisfaction with the dip tank activities (P<0.001) and the median (IQR) was -0.5 (-2 – 0) for the not-satisfied, 3 (2 – 4) for the little satisfied and 5 (5 – 7) for the very satisfied respondents. The median (IQR) vaccination perception score was 5 (2.7 – 5) for the non-formal education level, 5 (4 – 7) for primary level education and 5 (3.7 – 7) for the secondary level education and differences were not significant (P = 0.201).

3.2 Serological status of cattle

A total of 286 blood samples were collected from 2 herds each in 15 community dip tanks within the study area. The median (IQR) age for all the animals sampled was 4.5 (2.5-6.0) years and the median (IQR) period since most recent FMD vaccination was 189 (168-241) days. Relative to an antibody titre of ≥ 1.6 $\log_{10}$, seroprevalence adjusted for clustering and sampling factions were 20% (95%CI: 14-26), 39% (95%CI: 32-46) and 22% (95%CI: 17-27) to SAT 1-3, respectively. Median titres for each SAT serotype varied among herds and dip tanks (Figs 1 - 3). Seropositivity was less than 80% for all SAT serotypes in all but a single herd. One herd in Share Community had a marked serological response with 80% proportions

of cattle being seropositive for SAT 3. Herds in Share and Utha Scheme also had 50% and 60% of cattle seropositive for SAT 3 respectively. The retrospective review of the records of the previous mass vaccination campaign across all the study area indicated greater than 90% vaccination coverage.

Eighteen percent (95%CI: 10 - 29) of male and 21% (95%CI: 16 - 27) of female cattle were seropositive for SAT 1(Table 4; P = 0.575). Seropositivity was highest in animals older than 2 years, 22% (95%CI: 17 - 28) but age was not a significant predictor of SAT 1 serological status (P = 0.125). Brahman cattle tended to have lower SAT 1 seropositive proportions compared to Brahman cross and the local Nguni breed but the association was not significant (P = 0.102). Similar associations were estimated for SAT 2 and SAT 3 (Table 4).

4. Discussion

The aim of this study was to evaluate the current perceptions of communal farmers concerning FMD epidemiology, vaccination and control and also determine the proportions of cattle with presumed protective titres for FMD and herds with adequate herd immunity at a single location of the wildlife/livestock interface of the Kruger National Park. To our knowledge, this is the first survey regarding the perceptions of communal farmers concerning FMD control since the development of the Great Limpopo Transfrontier Conservation Areas (GLTFCA).

More respondents (76/104) were involved in herding their own cattle rather than employing paid handlers. Both men and women were involved in herding animals in this area with men accounting for 84% (87/104) of the respondents. In communal areas of South Africa, men and women share the responsibility of keeping livestock (Bester, et al., 2009). Communal farmers have been known to keep cattle for socio-cultural purposes including lobola

(compensation to the family of the bride prior to a wedding ceremony) and to settle disputes (compensation for damages) in communal areas (Chimonyo, et al., 1999).

The majority of farmers (64%) indicated that pregnant animals should not be vaccinated and this is a possible factor that would limit participation in a vaccination campaign. Sick animals, however, were not perceived as a reason to not present animals for vaccination. No other evaluated factors were perceived to affect farmers' presentation of cattle for vaccination. Livestock are important to communal farmers for special ceremonial gatherings such as marriage feasts, weddings, funerals and circumcision (Bayer, et al., 2004) suggesting that married households might have larger herd sizes. Although not statistically significant, married households actually had descriptively smaller herd sizes when compared to other categories.

Seventy-four percent of the respondents had either primary or secondary education qualifications indicating that the majority of farmers are literate and therefore more likely to adopt innovations. However, the majority of non-satisfied individuals had high education qualifications. This suggests that more educated farmers perceived inadequacies in the current animal health programmes and more education was descriptively associated with large vaccination perception scores.

FMD is considered the most important livestock disease at the interface (Vosloo, et al., 2002b; Vosloo, et al., 2002a; Thomson and Bastos, 2004), yet farmers do not have extensive knowledge of the disease. In this study, only 12% of the respondents indicated knowledge of any disease causing lesions similar to FMD when described in the local language. This suggests that despite the fact that efforts are in place for the control of FMD at the interface few farmers have adequate knowledge. Therefore, there is a need for educational programmes concerning FMD and other important livestock diseases among communal farmers in

addition to the current programmes. The high number of respondents (96%) indicating that they contact a veterinarian for disease situations is a reflection of the current animal health awareness within the study community and veterinarians could be an important source of educational information.

With the global increase in the human population, there is a need to improve livestock production across the entire livestock industry. Beef is in high demand for export markets on the basis of taste and texture (Delgado, et al., 1999; Chadwick, et al., 2008). However, some areas in Africa do not have sufficient beef to feed the local populations (Albrechtsen, et al., 2005). Other animals raised in addition to cattle in this area include pigs, goats, and chickens. Goats are herded together with cattle in many communal areas of South Africa (Bester, et al., 2009) and are not routinely vaccinated against FMD. The presence of small ruminants could therefore be a risk factor for the occurrence or extended propagation of outbreaks. Respondents indicated that wildlife increase the risk for disease in livestock at water points and shared grazing. Furthermore, the African buffalo was reported by the majority of respondents (92%) as representing a risk for disease outbreaks in cattle. Contacts between livestock and wildlife reservoirs have been previously reported to occur at this interface (Brahmbhatt, et al., 2012). These findings indicate that some knowledge concerning FMD epidemiology has been transferred to the local community.

Cattle in the Mnisi communal area are routinely vaccinated using an inactivated FMD vaccine containing SAT 1, SAT 2 and SAT 3 serotypes. However, the proportion of cattle with high levels of detectable antibody was low suggesting that the area is at risk for the active spread of FMD virus. This finding is consistent with previous research where the antibody level induced by alhydrogel-saponin SAT type vaccine preparation fell below 1.6 $\log_{10}$ VNT titre between 2 and 3 months after inoculation (Hunter, 1996). However, in

another study using a trivalent double emulsion vaccine, antibody levels to all SAT serotypes were maintained at >1.6 $\log_{10}$ for eleven months post-vaccination (Hunter, 1996).

FMD outbreaks have been reported in the protection zones of the Limpopo and Mpumalanga provinces and the KNP since 2008. Serotype SAT 1 was responsible for 4 outbreaks in 2009, 4 outbreaks in 2010 and 45 outbreaks in 2011. The 2011 outbreaks occurred in disease free zones of Kwazulu Natal and Gauteng provinces. SAT 2 was responsible for 2 outbreaks in 2008, 4 outbreaks in 2010, 2 outbreaks in 2011 and 5 outbreaks in 2012. SAT 3 was responsible for 1 outbreak in KNP wildlife in 2008 (WAHID, 2013).

In this study, we used heterologous antigens to test for FMD-specific antibodies in sera from vaccinated cattle using liquid-phase blocking ELISA because the viruses included in the commercial vaccine is proprietary information. This might be an explanation for the high variability in measured titres observed for SAT 1 and SAT 3. Since cattle in the protection zones are routinely vaccinated for FMD and all animals were greater than 6 months of age, it was assumed that all sampled cattle had received at least a single vaccination prior to the study. In the absence of reported outbreaks, measured antibody titres against FMDV structural proteins were suggestive of a vaccinal response rather than previous exposure to field virus.

The majority of sampled cattle were females and the SAT 1 antibody response was descriptively greater than sampled bulls. Female cattle form the majority of the cattle population within communal areas and farmers might present more female cattle for vaccination because bulls are more difficult to handle at the dip tanks. Serotype SAT 2 antibody titres appeared to be more consistent relative to the SAT 1 and SAT 3 titres and this might suggest a closer antigenic relationship between the vaccine strain and the test antigen. This could also explain why more herds had seropositive proportions greater than 50% for

SAT 2 antibodies compared to the other serotypes. SAT 2 viruses have been reported to have more sequence variation in the VP1 gene relative to other serotypes and vaccine manufacturers often select immunodominant vaccine strains with broad antigenic coverage (Rweyemamu, 1978). The antibody response to SAT 3 was relatively poor in adult cattle and demonstrated high variability between herds and dip tanks (similar to SAT 1). However, there were two herds at Utha Scheme that had 50% and 60% seropositivity and a herd at Share with 80% seropositivity. This could be related to variable antigenic stability within multivalent FMD vaccines but might also indicate virus circulation in the absence of clinical disease.

Age was not a significant predictor for seropositivity contrary to our expectations that older cattle would have large seropositive proportions relative to younger calves because of exposure to repeated immunisations. This observation could be due to a rapid antibody decline and poor stimulation of memory B-cells. Sex was not a significant predictor for any of the SAT type FMD virus but breed appeared to be important for SAT 1 and SAT 3. Fewer Brahman cattle were seropositive for SAT 1 and SAT 3 and even though the difference was not significant, might indicate that the local Nguni breed have better immune responses to vaccination. Genetic markers of disease resistance and productivity for this breed have been reviewed elsewhere (Scholtz, 1988; Mapiye, et al., 2007).

FMD vaccines predominantly stimulate a humoral immune response in cattle and there is a strong correlation between antibody levels and protection against challenge with homologous field virus (Ahl, et al., 1983; Sutmöller, et al., 1983; Pay and Hingley, 1987). Therefore serological evidence of FMD antibodies in vaccinated animals in the absence of circulating field virus is an indicator of protection to field challenge. The low proportion of seropositivity observed might be an indication of rapidly declining humoral response and

reduced protection, which is consistent with reports that aqueous FMD vaccines stimulates a short-lived duration of immunity (Hunter, 1998).

This study is limited to information obtained from livestock owners and handlers using an interview questionnaire and did not collect information related to the economic aspects of FMD vaccination and control. Respondents might not have presented their true views because of the nature of the in-person interview. Selection bias might have also occurred based on the convenient selection of respondents for the interview. Therefore, future studies should include the views of all stakeholders (veterinarians and animal health care workers) involved in vaccine administration. The small number of young cattle sampled might have limited our understanding of the immune status of young animals in this study. Also the fact that cattle were selected based on the convenient sampling of farmers at dip tanks might have generated incomplete representation of the target population. The incomplete sampling of cattle within the herd is also another limitation that affects inferences concerning the proportion of seropositive cattle within each herd. The use of heterologous antigens within the liquid-phase blocking ELISA might have underestimated the proportion of cattle classified as seropositive.

5. Conclusion

Owing to the increased occurrence of FMD outbreaks in recent time despite sustained efforts in routine prophylactic vaccination of cattle at the interface, there is a need to develop a cost effective vaccination programme that will induce effective herd immunity. The perception of livestock owners concerning a disease control intervention (e.g. FMD vaccination) is critical because this perception affects their decision to adopt a new technology or innovation (Adesina and Baidu-Forson, 1995). Education has been observed to be a factor that could

influence the perceptions of farmers and so this should be considered when designing progressive control programme at the interface.

Conflict of interest

None

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Table 15. The association between levels of satisfaction with dip tank activities and potential categorical predictors.

		Not satisfied (n=10)		Little satisfied (n=26)		Very satisfied (n=68)		
Variable	**Total**	**Frequency**	**% (95%CI)**	**Frequency**	**% (95%CI)**	**Frequency**	**% (95%CI)**	**P-value***
Description of respondents								
Owner	76	8	80 (48-97)	21	81 (62-93)	47	69 (57-79)	0.456
Gender								
Male	87	9	90 (60-100)	21	81 (62-93)	57	84 (74-91)	0.796
Marital status								
Single	27	3	30 (8-62)	5	19 (7-38)	19	28 (18-39)	0.658
Married	57	6	60 (29-86)	15	58 (38-75)	36	53 (41-65)	0.864
Divorced/Widowed	21	1	10 (0.5-40)	8	31 (15-50)	12	18 (10-28)	0.651
Most important source of income								
Livestock	103	9	90 (60-100)	26	100 (89-0)	68	100 (96-100)	0.090
Crop	11	0	0 (0-26)	6	23 (10-42)	5	7 (3-16)	0.044
Other animals kept								
Pig	13	1	10 (0.5-40)	6	23 (10-42)	6	9 (4-17)	0.168
Goat	40	3	30 (8-62)	11	42 (25-62)	26	38 (27-50)	0.791
Chicken	78	6	60 (29-86)	21	81 (62-93)	51	75 (64-84)	0.435
Source of drinking water								
Pipe	3	0	0 (0-26)	2	8 (1-23)	1	1 (0-7)	0.231
Well	85	8	80 (48-97)	20	77 (58-90)	57	84 (74-91)	0.732
Pond	19	2	20 (4-52)	6	23 (10-42)	11	16 (9-26)	0.732
FMD history								
Suspected previous outbreak	12	2	20 (4-52)	2	8 (1-23)	8	12 (6-21)	0.582
Disease management practices								
Contacting a veterinarian	100	7	70 (38-92)	26	100 (89-0)	67	99 (93-100)	0.002
Self-treatment	12	5	50 (21-79)	3	12 (3-29)	4	6 (2-14)	<0.001
Grazing management								
Contact with wildlife	20	1	10 (0.5-40)	4	15 (5-33)	15	21 (12-31)	0.563
Grazing adjacent to the Park	53	3	30 (8-62)	13	50 (31-69)	37	54 (43-66)	0.351

CI = confidence interval. FMD = foot-and-mouth disease

*Based on Fisher exact and chi-square tests

Table 16. The association between the levels of satisfaction with dip tank activities and potential continuous predictors

	Not satisfied		Little satisfied		Very satisfied		
Variable	**N**	**Median (IQR)**	**n**	**Median (IQR)**	**N**	**Median (IQR)**	**P-value***
Age of respondents (years)	8	31 (25-53)	22	56 (36-67)	59	45 (33-67)	0.065
Level of education	10	9 (2-12)	24	3 (0-6)	66	7 (2-11)	0.036
Time since last purchase of cattle (years)	2	2	7	4 (2-11)	21	4 (2-19)	0.407
Time since last sale of cattle (years)	5	1 (1-2)	8	1 (1-1)	31	2 (1-2)	0.055
Time since last introduction of new stock (years)	2	2	4	2 (2-3)	7	2 (1-2)	0.272
Duration in livestock farming (years)	5	22 (9-40)	13	17 (8-37)	42	12 (5-23)	0.487
Daily grazing distance (km)	10	3 (2-5)	26	4 (2-5)	68	4 (2-5)	0.635
Number of cattle owned by respondents	10	11 (6-27)	26	7 (6-19)	68	12 (5-19)	0.639
Number of herds owned by respondents	10	1 (1-1)	26	1 (1-1)	68	1 (1-1)	0.866
Frequency of annual FMD vaccination	10	2 (2-2)	26	2 (2-3)	68	2 (2-3)	0.666

IQR = Interquartile range

*Based on Kruskal-Wallis tests

Table 17. Frequency of responses to questions that could affect farmers' participation in vaccination programs

	Yes		No		Unsure	
Question	**Percent (n)**	**Score**	**Percent (n)**	**Score**	**Percent (n)**	**Score**
Vaccination can reduce disease in cattle	90 (94)	1	2 (2)	-1	8 (8)	0
Vaccination can make cattle sick	10 (10)	-1	72 (75)	1	18 (19)	0
Vaccination can cause abortion in cattle	14 (15)	-1	75 (78)	1	11 (11)	0
Vaccination improves cattle wellbeing	92 (96)	1	6 (6)	-1	2 (2)	0
Vaccination decreases feed intake in cattle	4 (4)	-1	90 (94)	1	6 (6)	0
Should sick cattle be presented for vaccination?	98 (102)	1	2 (2)	-1		
Should pregnant cattle be presented for vaccination?	35 (36)	1	64 (67)	-1		

Table 4. Serological responses to SAT 1, SAT 2 and SAT 3 on the basis of sex, age and breed (titre ≥1.6 Log_{10})

Serotype	Variable	Total	No. positive	Percentage (95%CI)	P-value*
SAT 1	Overall	286	58	20 (14 – 26)	
	Sex				
	Male	62	11	18 (10 – 29)	0.575
	Female	224	47	21 (16 – 27)	
	Age				
	≤12 months	18	4	22 (7 – 45)	0.125
	13-24 months	38	3	8 (2 – 20)	
	≥24 months	230	51	22 (17 – 28)	
	Breed				
	Brahman (typical)	46	4	9 (2 – 20)	0.102
	Brahman cross	108	24	22 (15 – 31)	
	Nguni	132	30	23 (16 – 30)	
SAT 2	Overall	286	109	39 (32 – 46)	
	Sex				
	Male	62	25	40 (29 – 53)	0.686
	Female	224	84	38 (31 – 44)	
	Age				
	≤12 months	18	3	17 (4 – 39)	0.143
	13-24 months	38	14	37 (23 – 53)	
	≥24 months	230	92	40 (34 – 46)	
	Breed				
	Brahman (typical)	46	14	30 (18 – 45)	0.155
	Brahman cross	108	37	34 (26 – 44)	
	Nguni	132	58	44 (36 – 52)	
SAT 3	Overall	286	68	22 (17 – 27)	
	Sex				
	Male	62	11	18 (10 – 29)	0.208
	Female	224	57	25 (20 – 31)	
	Age				
	≤12 months	18	5	28 (11 – 51)	0.117
	12-24 months	38	4	11 (3 – 23)	
	≥24 months	230	59	26 (20 – 32)	
	Breed				
	Brahman (typical)	46	5	11 (4 – 22)	0.062
	Brahman cross	108	26	24 (17 – 33)	
	Nguni	132	37	28 (21 – 36)	

*Based on chi-square or Fisher exact tests.

CI = Confidence interval

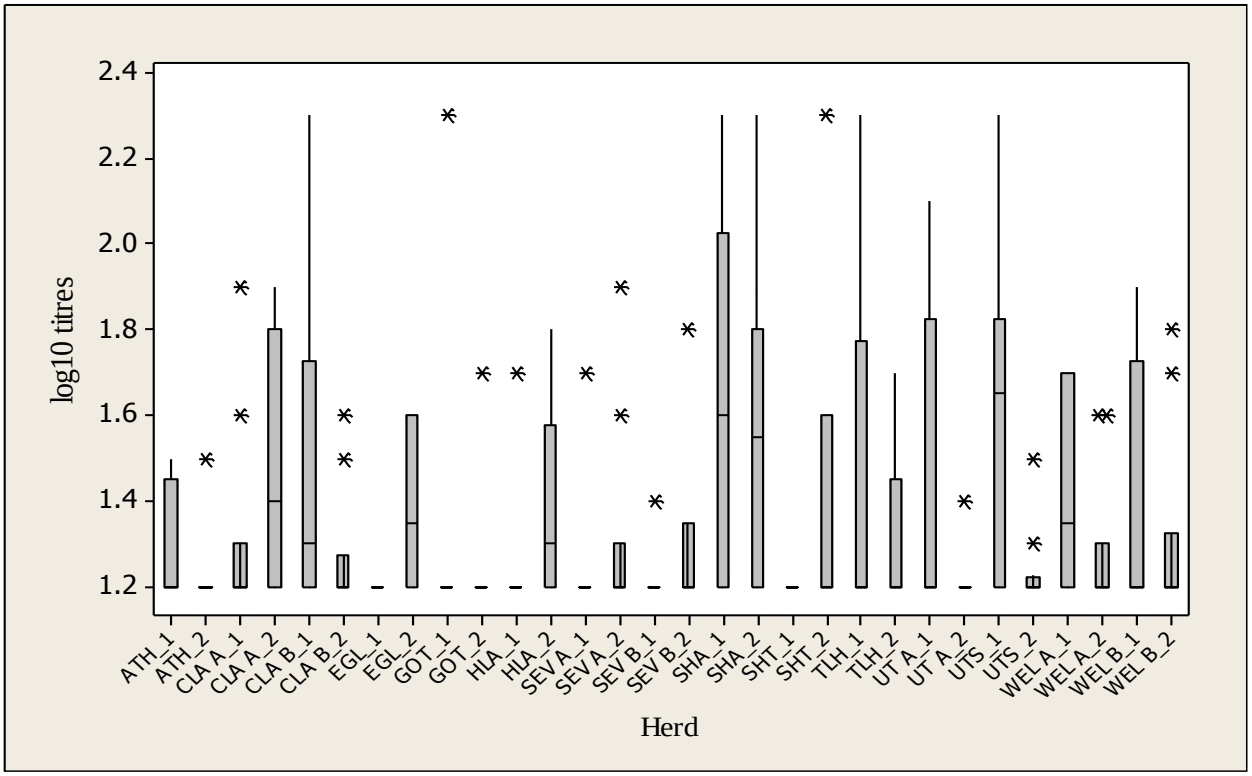

Figure 6. SAT 1 distribution by herd

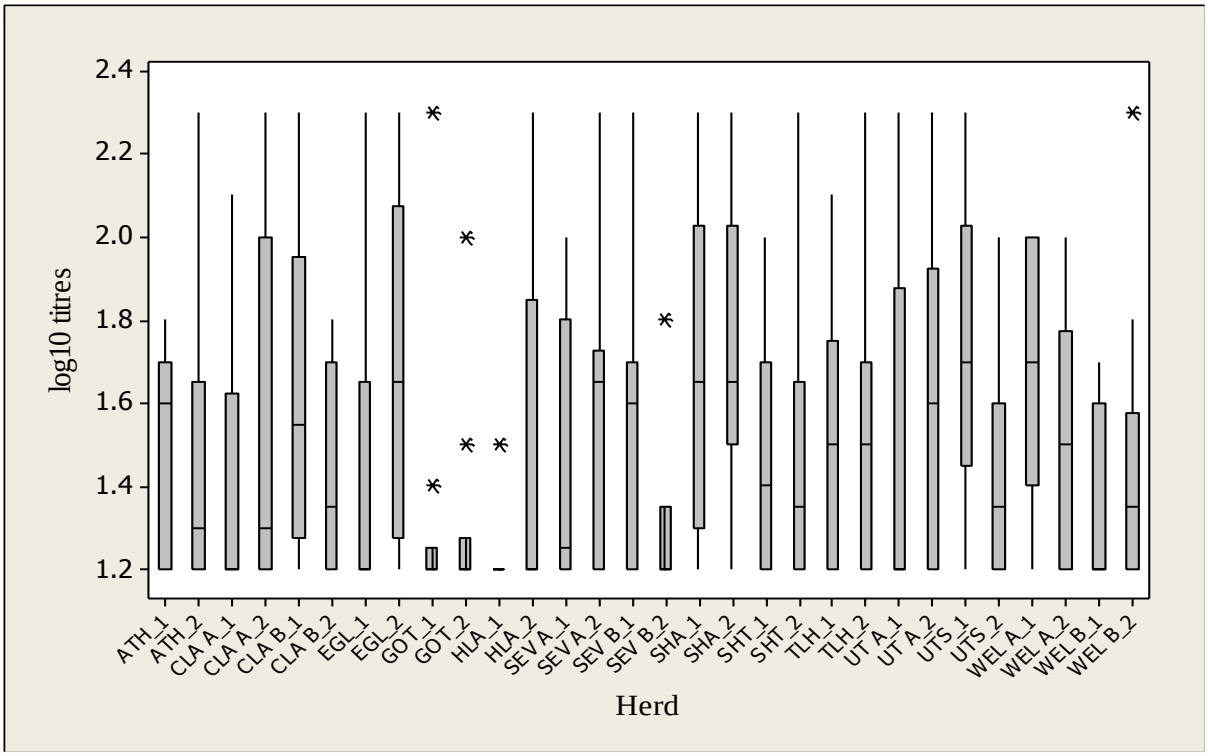

Figure 7. SAT 2 titre distribution by herd

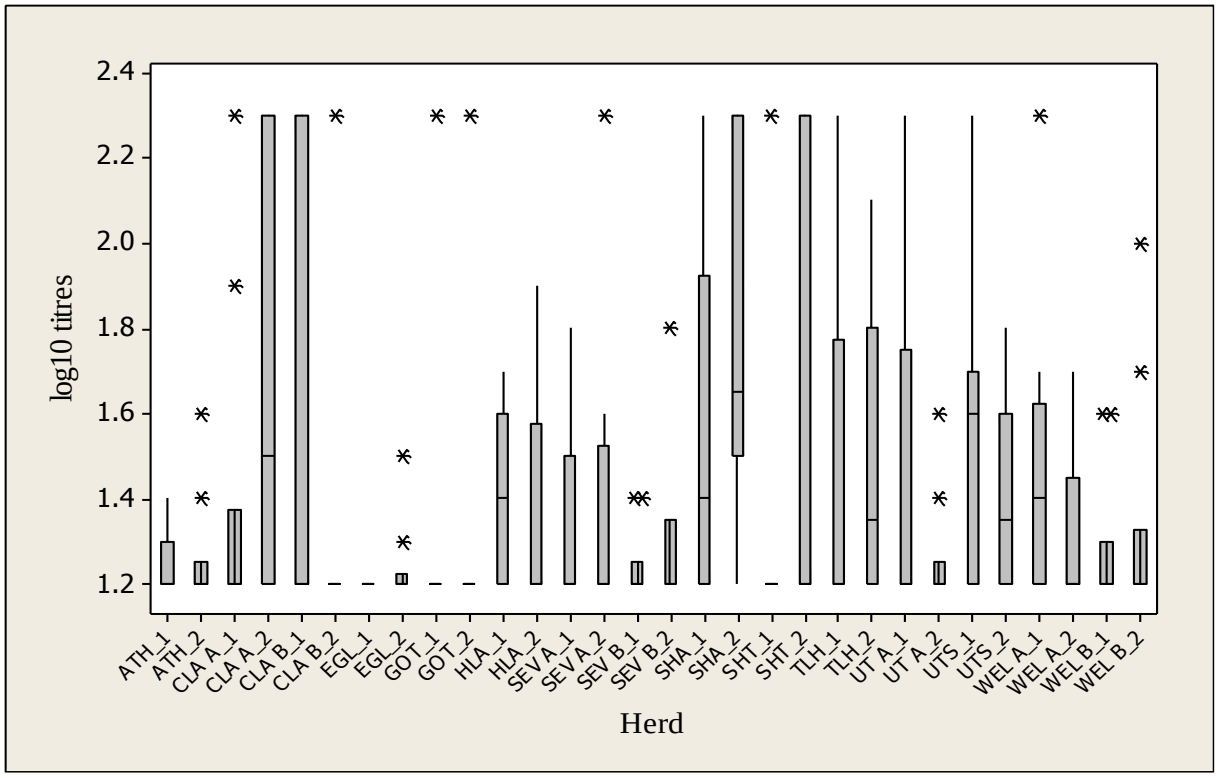

Figure 8. SAT 3 titre distribution by herd

APPENDIX III

MANUSCRIPT II

Serological responses and duration of titres in cattle inoculated with an inactivated trivalent Foot-and-mouth disease vaccine

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Abstract

Foot-and-mouth disease virus (FMDV) is one of the world's most important animal pathogen, responsible for losses in livestock trade, as well as frequent and highly disruptive large-scale epidemics. The control of FMD in southern Africa typically includes vaccination with a trivalent or bivalent vaccine preparation depending on the country. The objective of this study was to determine the duration of the humoral immune response conferred by the current FMD vaccination programme in cattle at the wildlife/livestock interface of the Kruger National Park (KNP) in South Africa. Two hundred and ninety-three cattle from 4 community dip tanks at the wildlife interface region of the KNP were longitudinally followed for a total of four months after vaccination with trivalent FMD vaccine (SAT 1, SAT 2 & SAT 3). Blood samples were collected every 2-weeks and vaccinal antibodies were measured using a liquid-phase blocking ELISA. The majority of cattle seroconverted ($\log_{10}$ titre $\geq$1.6) by 14 days post-vaccination with the highest proportions for SAT 2. Antibody responses remained at a relatively high level through 42 days but at 56 days post-vaccination, the proportion of seropositive cattle declined to less than 50% for all three serotypes. By the end of the four-month observation period, no location had a seropositive percentage of at least 80% seropositive cattle for any serotype. Measured antibody titres varied by serotype ($P < 0.001$, sex ($P = 0.018$) and age ($P < 0.001$). Cattle 6-12 months of age had lower serological responses to vaccination compared to older cattle. The duration of the antibody response to the current vaccination programme was less than 4-months and therefore the current 4-6 months inter-vaccination interval appears to be too long for use with the current vaccine. More research is necessary to determine the reasons for the limited duration of vaccinal antibodies in effort to limit the number of outbreaks at the wildlife interface of Kruger National Park.

Keywords: Cattle, Foot-and-mouth disease, serology, vaccination, wildlife interface

1. Introduction

Foot-and-mouth Disease virus (FMDV) naturally infects cloven-hoofed species and camelids, and causes an acute illness characterised by fever and lesions in the oral cavity, coronary band, interdigital space and teats (Kitching, 2002b). 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 response in both vaccinated and non-vaccinated animals. FMDV structural proteins stimulate the production of neutralising antibodies that provide protection against future disease challenges. Antibodies against non-structural proteins do not offer clinical protection (Grubman and Baxt, 2004).

Seven immunologically distinct FMDV types have been described, namely serotypes A, O, C (the so-called European types), Asia -1 and the three South African Territories (SAT) types 1, 2 and 3. Serotypes A, O, C and Asia-1 constitute a distinct lineage separate from the SAT viruses (Vosloo, et al., 2009). This serological classification is based on the inability of the viruses from different serotypes to induce cross protection in animals (Pereira, 1976). However, subsequent research findings have demonstrated antigenic variation within FMDV serotypes (Mateu, et al., 1988; Samuel, et al., 1990; Samuel and Knowles, 2001; Tosh, et al., 2003).

Foot-and-mouth disease (FMD) causes large economic effects on trade at the local, national and international levels and substantial funds are invested worldwide for prevention and control (OIE, 2012b). Routine vaccination of susceptible cloven hoofed livestock with inactivated FMD vaccine is recommended for control in endemic countries. The OIE classifies FMD vaccines as either "standard" or "high potency" vaccines based on the quantity of antigen. A standard vaccine with a potency of $3PD_{50}$ (three-times the dose of

vaccine that protects 50% of the challenged animals) and appropriate adjuvant is considered suitable for routine vaccination campaigns in FMD endemic locations (Elnekave, et al., 2013). Most FMD vaccines in endemic countries often contain more than one virus serotype, depending upon the epidemiological situation of the particular country. In southern Africa, the employed vaccines are typically trivalent (SAT 1-3) or bivalent (SAT 1 & 2) depending on the country (Thomson and Bastos, 2004).

Serological assays including virus neutralisation tests (VNT) and enzyme-linked immunosorbent assay (ELISA) have been employed to measure serological responses to vaccination in cattle (Sutmoller and Vieira, 1980; McCullough, et al., 1992b), with good correlation between antibody titres and protection to challenge with a live virus (Pay and Hingley, 1987). FMD vaccines frequently applied for prophylactic use in endemic settings have been reported to provide immunity for a period of 4-6 months in the absence of regular booster doses (Doel, 2003). However, despite a good correlation between serum antibody titres and protection, there are instances where animals with substantial antibody titres are not protected from disease after experimental challenge (McCullough, et al., 1992a). Similarly, animals with low or no detectable antibody do not always succumb to disease (Sobrino, et al., 2001).

FMD viruses demonstrate extensive antigenic variability and a vaccine prepared from one isolate will not necessarily provide protection against infection with another field virus of the same serotype (Fargeaud, 1995). There is a need to match field viruses to the available reference strains to select a vaccine with expected utility within the target region. FMD control programmes should utilise vaccines developed from representative field isolates to adequately prevent outbreaks (Paton, et al., 2005). The performance of vaccines containing FMDV serotypes O, A and Asia 1 has been studied extensively (Pay and Hingley, 1987; Guo, et al., 2005; Eblé, et al., 2006; Chen and Liu, 2013; Lee, et al., 2013), however, little is

known about the field performance of inactivated FMD vaccines containing serotypes SAT 1, SAT 2 and SAT 3.

The objective of this study was to determine the duration of the humoral immune response conferred by the current FMD vaccination programme in cattle at the wildlife interface of the Kruger National Park in South Africa.

2. Materials and methods

2.1 Ethical clearance

The study was approved by the Animal Ethics Committee (Project Number V010-12) at the University of Pretoria, Faculty of Veterinary Science. Section 20 approval was obtained from the Animal Health Directorate: Department of Agriculture, Forestry and Fisheries (Application Number 12/11/1/1) of the Republic of South Africa.

2.2 Description of the study area

Mnisi Community is a communal area situated within the FMD protection zone with vaccination adjacent to the Kruger National Park (KNP) in Mpumalanga Province of the Republic of South Africa. One of the major activities of the residents of this community is livestock herding that typically employs an extensive free range system. Cattle are routinely vaccinated for FMD virus using a trivalent vaccine containing SAT 1, SAT 2 and SAT 3 antigens. More details concerning the study location have been presented elsewhere (unpublished Lazarus, et al., manuscript #1).

2.3 Sample size justification

The sample size calculations were performed to estimate the proportion of cattle with ≥1.6 $\log_{10}$ titres (seropositive) at any sampling period post-vaccination. It was assumed that 80% of cattle would become seropositive and it was desired to estimate this proportion +/- 10% at

the 95% level of confidence. A design effect of 4 was assumed to account for the clustering of cattle within dip tanks and also within herds. The sample size was estimated as 246 cattle based on these assumptions.

2.4 Selection of cattle

Four community dip tanks from a list of 16 communities in the Mnisi communal area were purposively selected based on the scheduling of weekly dipping sessions. The four dip tanks were selected to represent 2 dip tanks each from two wards managed by different animal health technicians. At each dip tank, seven herds were conveniently selected after obtaining informed consent from farmers concerning the necessary 4 month follow-up. Ten cattle greater than 6 months of age were purposely selected from each participating herd. The age of enrolled cattle was determined based on dentition and available information from the herder and subsequently categorised as 6-12 months, 13-24 months and >24 months. Ear tags were applied to all selected cattle for identification purposes.

2.5 Vaccination procedure

Provincial government veterinary services performed the routine FMD mass vaccination programme during June 2012. Cattle were vaccinated subcutaneously in the neck region using an automated syringe system. Each animal was injected with 5 ml of a commercial aqueous aluminium hydroxide and saponin-adjuvanted inactivated trivalent FMD vaccine containing SAT 1, SAT 2 and SAT 3 strains (Aftovac®, Merial Animal Health Ltd/Botswana Vaccine Institute Gaborone). The vaccine batch number was 13309 and had a December 2012 expiry date.

2.6 Specimen collection

Blood samples were collected on the day of vaccination (Round 0) and at 2-week intervals over a 4-month follow-up period (Rounds 1-8). Whole blood samples were collected from the

mid-coccygeal or jugular vein into 10 ml plain evacuated tubes (Vacutainer® tubes, Becton, Dickinson and company, Franklin Lakes, New Jersey, USA). Blood was allowed to clot at ambient temperature in the field and transported to the laboratory within 6 hours of collection. Blood was centrifuged at 1450 g for 10 min immediately after delivery to the laboratory. Serum was decanted into sterile cryovials and stored at -20˚C until testing. Sera were packaged according to the Regulations of the National Road Traffic Act, 1996 (Act No. 93 of 1996) of the Republic of South Africa and transported on ice to the Transboundary Animal Disease Programme Laboratory of the Onderstepoort Veterinary Institute (TADP), Pretoria for testing.

2.7 Laboratory testing

Samples were analysed for FMD-specific antibodies using a liquid phase blocking ELISA as previously described by Hamblin, et al. (1986b). Assays were performed using an in-house developed ELISA kit for SAT 1, SAT 2 and SAT 3. This test is based upon serotype specific blocking of liquid phase FMD antigen by antibodies in the test serum sample. Antibody titres were expressed as the 50% end-point titres and sera with titres ≥ 1.6 $\log_{10}$ were classified as seropositive.

2.8 Statistical analysis

The percentage of seropositive cattle (≥ 1.6 $\log_{10}$ titre) at each round of bleeding was determined and 95% confidence intervals (CI) were calculated using the exact mid-P method. Quantitative data were described using medians and interquartile ranges (IQR). Kruskal-Wallis (KW) tests were used to compare titre data over groups of potential predictors. A linear mixed model was fit to estimate the effect of covariates on measured titres. Models included random effects for dip tank and herd and evaluated fixed effect terms for sampling round, serotype, age category, sex, breed, and an interaction between serotype and sampling round. A backwards step-wise approach was used to fit a final main effects model and

biologically plausible pairwise interaction terms were added one-by-one to test for effect measure modification. Descriptive data analysis was performed using one statistical package (Minitab, Version 16 State College, PA, USA) and the linear mixed model in another (IBM SPSS Statistics Version 21, International Business Machines Corp., Armonk, New York, USA). Results were interpreted at the 5% level of significance.

3. Results

A total of 293 cattle were sampled at 4 community dip tanks during the 112 day study period. Complete follow-up was not obtainable for all selected cattle (Table 1). At the commencement of the study, few cattle were seropositive (≥1.6 $\log_{10}$ titre) for SAT 1 & 3 viruses (Table 2). However, 14 days post-vaccination, the proportion of seropositive cattle to the three SAT type virus was between 66% - 91% with SAT 2 having the highest proportions. Twenty-eight days after vaccination, the corresponding proportions of cattle with a titre >1.6 $\log_{10}$ ranged between 45% - 75% for SAT 1, 45% - 75% for SAT 2 and 49% - 70% for SAT 3. Antibody responses remained high through 42 days post-vaccination but then started to decline. By the end of the 112 day follow-up, antibody responses to all serotypes was less than 80% at all study locations with SAT 2 having the highest proportions of (57% - 77%) per study location, SAT 1 the next highest (46% - 66% per location) and SAT 3 the lowest (28% - 42% per location).

The SAT 1 antibody titre varied by age (P = 0.015) at 42 days post-vaccination and by sex (P = 0.002) at 112 days post-vaccination (Table 3). SAT 2 titres varied by age (P = 0.025) at 42 days post-vaccination with cattle 6-12 months of age having lower titres compared to other age groups (Table 4). Female cattle had higher SAT 2 titres compared to male at 112 days post-vaccination (P = 0.004). SAT 3 titres varied by sex (P = 0.011), age (P = 0.002), breed (P = 0.033) and dip tank (P = 0.001) at 42 days post-vaccination (Table 5).

Overall, age was a significant predictor of antibody titre ($P < 0.001$) with cattle >13 months of age having higher titres than younger cattle (Table 6). Antibody titres varied by serotype with SAT 2 having higher titres ($P < 0.001$) and the duration of antibody responses varied by serotype ($P < 0.001$). Female cattle also had better serological responses compared to males ($P = 0.018$). The predicted $\log_{10}$ antibody levels peaked at 14 days post-vaccination for SAT 1, 42 days post-vaccination for both SAT 2 and SAT 3 (Table 7).

4. Discussion

The proportion of cattle with meaningful titres to previous vaccination was low suggesting that the interval between vaccinations was too long to maintain adequate vaccinal titres in the study area. However, by 14 days post-vaccination, a large proportion of the study population had seroconverted to all SAT serotypes indicating a serological response to vaccination. This is consistent with a previous study in which vaccinated cattle produced meaningful antibody titres between 14 and 28 days after a single vaccination (Doel, 2003). In a related study involving dairy herds in Saudi Arabia, it has also been demonstrated that maximum antibody titres are typically reached 7-10 days post-vaccination (Woolhouse, et al., 1996).

Duration of immunity is an important consideration for FMD vaccines (Hunter, 1998; Doel, 2003) and a vaccine that induces a strong serological response with a sustained duration is required for effective control (Cloete, et al., 2008). However, aqueous FMD vaccines are often unable to provide a sustained immunity in ruminants (exceeding 4-6 months). Thus, cattle in endemic areas require revaccination at regular intervals of 4-6 months to ensure protective levels of antibodies (Cox, et al., 2003). In this study, vaccination did not elicit sustained immune responses beyond 4 months for the majority of enrolled cattle. The critical inter-vaccination interval to prevent outbreaks could be unrealistically short when exposed to a heterologous field virus (Woolhouse, et al., 1996).

SAT 2 antibody responses were observed to be higher and last longer relative to SAT 1 and SAT 3. This however, is consistent with other studies in southern Africa that studied antibody responses in cattle vaccinated with a bivalent (SAT 1 &SAT 2) vaccine (Massicame, 2012). This observation could be due to a better antigenicity of the SAT 2 antigen or possibly a closer match of the SAT 2 vaccine strain with the antigen employed in the liquid-phase blocking ELISA used to estimate serological responses.

Age was a significant predictor of titre with adult cattle (>24 months of age) having higher antibody titres compared to younger cattle. This is not unexpected and is likely due to repeated exposures to the vaccine and subsequent anamnestic responses. Weanling cattle (6-12 months) had the lowest titres, which might have been caused by the interference of maternally derived antibodies (it is a common practice in the region to vaccinate very young calves during vaccination campaigns) at prior vaccination times or this being the first exposure of the animal to FMDV vaccine.

Female cattle had higher titres compared to male cattle. There is no biological explanation as to why female cattle might mount a better serological response since vaccination occurs at the same time and all animals are managed within the same system. A possible explanation is that female cattle might be presented for vaccination more regularly compared to male cattle due to the difficulty in handling and restraint during vaccination campaigns. It is also possible that inadequate injection might be more common in large bulls due to the difficulty in restraining the animal. The indigenous local Nguni breed formed the majority of the cattle population within Mnisi communal area, which is indicative of a traditional livestock production system. There was some evidence in the univariate analysis that breed might be an important predictor of SAT 3 titres, however, breed was not significant after entering into the multivariable random-effects model.

Location was not a significant predictor of titre when evaluated independently per sampling time and serotype (with an occasional exception) suggesting that there was not an important effect of veterinary technician and therefore important differences in vaccine administration. However, an overall effect of the veterinary technician could not be tested in the multivariable model since dip tank was included in the model as a random effect.

This study is limited by the fact that it only measured serological responses using a liquid-phase blocking ELISA based on heterologous antigens. The use of heterologous antigens in the liquid-phase blocking ELISA assay likely underestimated the proportion of cattle classified as seropositive relative to what would have been observed using a test employing homologous antigens. The lack of data related to virus neutralisation titres is another important limitation. For dip tank C and D, cattle were not sampled during the first week because of the on-going vaccination programme that did not permit simultaneous sampling of cattle at the dip tanks. Also, the study farmers could not present their cattle for sampling at dip tanks C & D because of excessive rainfall during the 48 day sampling period. Enrolled cattle were not available for complete follow-up because they were not always presented at the dip tanks during the scheduled sampling periods. The convenience sampling of farmers and cattle might have encouraged the enrolment of cattle systematically different than the general population of cattle (selection bias) and it is not possible to estimate the impact of this potential bias. Serological results were truncated at <1.2 and >2.2 $\log_{10}$ titres because of the standard laboratory procedures and this caused the distribution to be non-normal. Results of the random-effects model were consistent with the crude univariate results; however, the impact of this assumption violation could not be measured.

5. Conclusion

The current cattle vaccination programme at the wildlife/livestock interface of the Kruger National Park causes seroconversion in a high proportion of vaccinated cattle at 14 days post-vaccination. The duration of the humoral response, however was less than 4- months and therefore the current 4-6 month inter-vaccination intervals appears to be too long for use with the current. Young cattle might require repeated vaccination at a 2-4 week interval to guarantee adequate primary immunisation. In general, more research is necessary to determine the reasons for the limited duration of vaccinal antibodies in effort to limit the number of outbreaks at the wildlife interface of Kruger National Park.

Conflict of interest

None of the authors has financial or personal relationships that could influence or bias the content of the paper.

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Table 1. Number (%) of cattle sampled during the 4 month study period.

	Diptank				Age			Breed			Sex	
Day	A	B	C	D	6-12 mn	13-24 mn	>24 mn	Brahman	Brahman cross	Nguni	Female	Male
0	32 (42)	45 (58)	0 (0)	0 (0)	6 (8)	29 (38)	42 (55)	26 (34)	24 (31)	27 (35)	62 (81)	15 (19)
14	63 (28)	47 (21)	70 (31)	44 (20)	35 (16)	70 (31)	119 (53)	24 (11)	98 (44)	102 (46)	162 (72)	62 (28)
28	59 (26)	44 (20)	65 (29)	56 (25)	33 (15)	72 (32)	119 (53)	30 (13)	99 (44)	95 (42)	156 (70)	68 (30)
42	50 (22)	51 (22)	71 (31)	59 (26)	33 (14)	73 (32)	125 (54)	21 (9)	108 (47)	102 (44)	167 (72)	64 (28)
56	65 (35)	0 (0)	66 (36)	53 (29)	33 (18)	55 (30)	96 (52)	21 (11)	79 (43)	84 (46)	130 (71)	54 (29)
70	65 (30)	38 (17)	68 (31)	49 (22)	29 (13)	66 (30)	125 (57)	26 (12)	88 (40)	106 (48)	162 (74)	58 (26)
84	28 (39)	44 (61)	0 (0)	0 (0)	7 (10)	26 (36)	39 (54)	14 (19)	21 (29)	37 (51)	53 (74)	19 (26)
98	34 (17)	49 (25)	51 (26)	66 (33)	33 (17)	58 (29)	109 (55)	21 (11)	89 (45)	90 (45)	146 (73)	54 (27)
112	65 (27)	54 (23)	57 (24)	64 (27)	36 (15)	75 (31)	129 (54)	31 (13)	97 (40)	112 (47)	180 (75)	60 (25)
Total	461 (28)	372 (22)	448 (27)	391 (23)	245 (15)	524 (31)	903 (54)	214 (13)	703 (42)	755 (45)	1218 (73)	454 (27)

Table 2. Percentage of cattle with titre ≥1.6 $\log_{10}$ according to study location and serotype.

Day	N	Dip tank	SAT 1 % (95% CI)	SAT 2 % (95% CI)	SAT 3 % (95% CI)
0	32	A	3 (0 – 14)	36 (22 – 55)	16 (6 – 31)
	45	B	7 (2 – 17)	47 (33 – 61)	16 (7 – 28)
	0	C			
	0	D			
14	63	A	81 (70 – 89)	84 (74 – 92)	73 (61 – 83)
	47	B	85 (73 – 93)	87 (75 – 95)	70 (56 – 82)
	70	C	86 (76 – 93)	93 (85 – 97)	89 (79 – 95)
	44	D	66 (51 – 79)	91 (80 – 97)	89 (77 – 96)
28	59	A	51 (38 – 63)	53 (40 – 65)	49 (37 – 62)
	44	B	45 (31 – 60)	45 (31 – 60)	55 (40 – 69)
	65	C	63 (51 – 74)	68 (56 – 78)	65 (52 – 75)
	56	D	75 (62 – 85)	75 (62 – 85)	70 (57 – 81)
42	50	A	86 (74 – 94)	100 (94 – 100)	88 (77 – 95)
	51	B	92 (82 – 97)	100 (94 – 100)	94 (85 – 98)
	71	C	75 (64 – 84)	86 (76 – 93)	83 (73 – 91)
	59	D	61 (48 – 73)	92 (82 – 97)	92 (82 – 97)
56	65	A	54 (42 – 66)	49 (37 – 61)	38 (27 – 51)
	0	B			
	66	C	68 (56 – 79)	58 (45 – 69)	47 (35 – 59)
	53	D	70 (57 – 81)	66 (53 – 78)	60 (47 – 73)
70	65	A	26 (17 – 38)	29 (19 – 41)	31 (20 – 42)
	38	B	26 (14 – 42)	37 (23 – 53)	24 (12 – 39)
	68	C	25 (16 – 36)	44 (33 – 56)	35 (25 – 47)
	49	D	33 (21 – 47)	45 (31 – 59)	47 (33 – 61)
84	28	A	29 (14 – 47)	25 (12 – 43)	36 (20 – 54)
	44	B	52 (38 – 67)	45 (31 – 60)	39 (25 – 54)
	0	C			
	0	D			
98	34	A	18 (7 – 33)	41 (26 – 58)	35 (21 – 52)
	49	B	37 (24 – 51)	45 (31 – 59)	41 (28 – 55)
	51	C	31 (20 – 45)	47 (34 – 61)	41 (28 – 55)
	66	D	48 (37 – 60)	53 (41 – 65)	61 (48 – 72)
112	65	A	52 (40 – 64)	57 (45 – 69)	28 (18 – 39)
	54	B	46 (33 – 60)	59 (46 – 72)	30 (19 – 43)
	57	C	53 (40 – 65)	77 (65 – 87)	26 (16 – 39)
	64	D	66 (53 – 76)	67 (55 – 78)	42 (31 – 55)

CI = confidence interval. NA = no animals sampled.

Table 3. Median and interquartile range (IQR) $\log_{10}$ antibody titre for SAT 1 by animal factors and location.

Day	Variable / level	N	Median (IQR)	P-value*
14	**Sex**			0.640
	Female	131	>2.20 (1.91, >2.20)	
	Male	50	>2.20 (1.76, >2.20)	
	Age			0.131
	6 – 12 months	23	1.93 (1.75, >2.20)	
	13 – 24 months	60	>2.20 (1.96, >2.20)	
	>24 months	98	>2.20 (1.87, >2.20)	
	Breed			0.590
	Brahman	21	>2.20 (1.74, >2.20)	
	Brahman cross	84	>2.20 (1.93, >2.20)	
	Nguni	76	>2.20 (1.88, >2.20)	
	Dip tank			0.854
	A	51	>2.20 (1.83, >2.20)	
	B	41	>2.20 (1.88, >2.20)	
	C	60	2.20 (1.92, >2.20)	
	D	29	2.20 (1.77, >2.20)	
42	**Sex**			0.191
	Female	136	>2.20 (1.88, >2.20)	
	Male	44	2.18 (1.80, >2.20)	
	Age			0.015
	6 – 12 months	23	1.88 (1.76, >2.20)	
	13 – 24 months	62	>2.20 (1.93, >2.20)	
	>24 months	95	>2.20 (1.87, >2.20)	
	Breed			0.580
	Brahman	16	2.10 (1.74, >2.20)	
	Brahman cross	79	>2.20 (1.87, >2.20)	
	Nguni	85	>2.20 (1.88, >2.20)	
	Dip tank			0.115
	A	43	1.97 (1.76, >2.20)	
	B	47	>2.20 (1.94, >2.20)	
	C	54	>2.20 (1.87, >2.20)	
	D	36	2.15 (1.87, >2.20)	
112	**Sex**			0.002
	Female	108	1.93 (1.73, 2.20)	
	Male	25	1.74 (1.67, 1.92)	
	Age			0.202
	6 – 12 months	12	1.75 (1.65, 2.08)	
	13 – 24 months	43	1.87 (1.72, >2.20)	
	>24 months	78	1.92 (1.73, >2.20)	
	Breed			0.096
	Brahman	17	1.77 (1.70, 1.92)	
	Brahman cross	60	1.85 (1.72, 2.26)	
	Nguni	56	1.95 (1.73, >2.20)	
	Dip tank			0.058
	A	34	1.77 (1.72, 2.18)	
	B	27	1.77 (1.70, 1.95)	
	C	30	1.92 (1.67, 2.03)	

D	42	2.06 (1.74, >2.20)

*Based on Mann-Whitney U tests for 2-group comparisons and Kruskal-Wallis tests for larger group numbers.

Table 4. Median interquartile range (IQR) log_{10} antibody titre for SAT 2 by animal factors and location.

Day	Variable / level	N	Median (IQR)	P-value*
14	**Sex**			0.535
	Female	147	>2.20 (>2.20, >2.20)	
	Male	52	>2.20 (>2.20, >2.20)	
	Age			0.021
	6 – 12 months	30	>2.20 (1.83, >2.20)	
	13 – 24 months	63	>2.20 (>2.20, >2.20)	
	>24 months	106	>2.20 (>2.20, >2.20)	
	Breed			0.392
	Brahman	20	>2.20 (>2.20, >2.20)	
	Brahman cross	92	>2.20 (>2.20, >2.20)	
	Nguni	87	>2.20 (>2.20, >2.20)	
	Dip tank			0.262
	A	51	>2.20 (>2.20, >2.20)	
	B	41	>2.20 (>2.20, >2.20)	
	C	60	2.20 (2.15, >2.20)	
	D	29	2.20 (>2.20, >2.20)	
42	**Sex**			0.445
	Female	158	>2.20 (>2.20, >2.20)	
	Male	58	>2.20 (>2.20, >2.20)	
	Age			0.025
	6 – 12 months	33	>2.20 (2.12, >2.20)	
	13 – 24 months	69	>2.20 (>2.20, >2.20)	
	>24 months	114	>2.20 (>2.20, >2.20)	
	Breed			0.606
	Brahman	20	>2.20 (>2.20, >2.20)	
	Brahman cross	101	>2.20 (>2.20, >2.20)	
	Nguni	95	>2.20 (>2.20, >2.20)	
	Dip tank			0.054
	A	50	>2.20 (>2.20, >2.20)	
	B	51	>2.20 (>2.20, >2.20)	
	C	61	>2.20 (>2.20, >2.20)	
	D	54	>2.20 (>2.20, >2.20)	
112	**Sex**			0.004
	Female	121	1.95 (1.75, >2.20)	
	Male	35	1.79 (1.69, 1.99)	
	Age			0.384
	6 – 12 months	20	1.80 (1.70, >2.20)	
	13 – 24 months	50	1.93 (1.75, >2.20)	
	>24 months	86	1.95 (1.75, >2.20)	
	Breed			0.356
	Brahman	18	1.97 (1.92, 2.20)	
	Brahman cross	67	1.87 (1.75, 2.20)	
	Nguni	71	1.94 (1.74, >2.20)	
	Dip tank			0.708
	A	37	1.91 (1.69, >2.20)	
	B	32	1.95 (1.77, >2.20)	

C	44	1.95 (1.75, >2.20)
D	43	1.89 (1.75, >2.20)

*Based on Mann-Whitney U tests for 2-group comparisons and Kruskal-Wallis tests for larger group numbers.

Table 5. Median and interquartile range (IQR) log10 antibody titre for SAT 3 by animal factors and location.

Day	Variable	N	Median (IQR)	P-value
14	**Sex**			0.555
	Female	131	>2.20 (1.95, >2.20)	
	Male	51	>2.20 (1.83, >2.20)	
	Age			0.198
	6 – 12 months	23	1.95 (1.75, >2.20)	
	13 – 24 months	58	>2.20 (1.96, >2.20)	
	>24 months	101	>2.20 (1.93, >2.20)	
	Breed			0.908
	Brahman	18	>2.20 (1.89, >2.20)	
	Brahman cross	84	>2.20 (1.95, >2.20)	
	Nguni	80	>2.20 (1.87, >2.20)	
	Dip tank			0.645
	A	62	>2.20 (1.86, >2.20)	
	B	46	>2.20 (1.86, >2.20)	
	C	40	2.20 (1.96, >2.20)	
	D	29	2.20 (1.96, >2.20)	
42	**Sex**			0.011
	Female	150	>2.20 (>2.20, >2.20)	
	Male	55	>2.20 (2.16, >2.20)	
	Age			0.002
	6 – 12 months	29	>2.20 (2.10, >2.20)	
	13 – 24 months	66	>2.20 (>2.20, >2.20)	
	>24 months	110	>2.20 (>2.20, >2.20)	
	Breed			0.033
	Brahman	19	>2.20 (1.96, >2.20)	
	Brahman cross	97	>2.20 (>2.20, >2.20)	
	Nguni	89	>2.20 (>2.20, >2.20)	
	Dip tank			0.001
	A	44	>2.20 (1.78, >2.20)	
	B	48	>2.20 (>2.20, >2.20)	
	C	59	>2.20 (>2.20, >2.20)	
	D	54	>2.20 (>2.20, >2.20)	
112	**Sex**			0.265
	Female	69	1.88 (1.73, >2.20)	
	Male	9	1.79 (1.63, 2.14)	
	Age			0.300
	6 – 12 months	4	2.11 (1.77, 2.35)	
	13 – 24 months	26	1.78 (1.65, 2.10)	
	>24 months	48	1.91 (1.73, >2.20)	
	Breed			0.150
	Brahman	9	1.79 (1.68, 2.11)	
	Brahman cross	35	1.84 (1.65, 1.97)	
	Nguni	34	1.94 (1.75, >2.20)	
	Dip tank			0.801
	A	18	1.83 (1.71, >2.20)	
	B	16	1.82 (1.67, >2.20)	

C	15	1.84 (1.66, 2.00)
D	29	1.93 (1.74, >2.20)

Table 6. Estimated marginal means for rounds, sex, age and virus serotype based on the mixed effect linear model that included random effects for dip tank and herd.

Variable / level	Mean log_{10} titre*	95%CI	P-value
Days			<0.001
0	1.34[a]	1.27 - 1.40	
14	1.97[b]	1.92 - 2.02	
28	1.71[b]	1.66 - 1.76	
42	2.06[b]	2.01 - 2.12	
56	1.65[b]	1.59 - 1.70	
70	1.45[b]	1.40 - 1.51	
84	1.53[b]	1.47 - 1.59	
98	1.55[b]	1.49 - 1.60	
112	1.57[b]	1.52 - 1.62	
Sex			0.018
Male	1.62	1.56 - 1.68	
Female	1.68	1.63 - 1.73	
Age category			<0.001
6-12 months	1.56[a]	1.49 - 1.63	
13-24 months	1.69[b]	1.63 - 1.75	
>24 months	1.70[b]	1.65 - 1.75	
Serotype			<0.001
SAT 1	1.61[a]	1.56 - 1.66	
SAT 2	1.71[b]	1.66 - 1.75	
SAT 3	1.63[a]	1.58 - 1.68	

CI = confidence interval.

*Means without suprascripts in common at statistically different after Bonferroni correction of P values.

Table 7. Means $\log_{10}$ titres (95% confidence interval) estimated from a mixed-effect linear model that included random effects for dip tank and herds.

Days	SAT 1	SAT 2	SAT 3
0	1.20 (1.12 – 1.28)	1.49 (1.42 – 1.58)	1.32 (1.23 – 1.39)
14	1.91 (1.85 – 1.97)	2.06 (2.00 – 2.12)	1.93 (1.87 – 1.99)
28	1.70 (1.64 – 1.76)	1.69 (1.64 – 1.76)	1.74 (1.68 – 1.79)
42	1.89 (1.84 – 1.96)	2.19 (2.13 – 2.25)	2.11 (2.10 – 2.17)
56	1.69 (1.64 – 1.76)	1.65 (1.59 – 1.71)	1.59 (1.54 – 1.66)
70	1.40 (1.34 – 1.46)	1.48 (1.42 – 1.54)	1.47 (1.41 – 1.53)
84	1.59 (1.51 – 1.67)	1.52 (1.44 – 1.59)	1.49 (1.40 – 1.57)
98	1.48 (1.42 – 1.54)	1.57 (1.51 – 1.63)	1.60 (1.54 – 1.66)
112	1.59 (1.54 – 1.66)	1.69 (1.63 – 1.74)	1.43 (1.37 – 1.49)