# Serological responses of cattle inoculated with inactivated trivalent foot-and-mouth disease vaccine at the wildlife-livestock interface of the Kruger National Park, South Africa

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## Abstract

Foot-and-mouth disease (FMD) virus is economically one of the world's most important animal pathogens, which can be 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 of cattle with a trivalent or bivalent vaccine preparation. The objective of this study was to determine the level and duration of the antibody response conferred by the current FMD vaccination programme in cattle at the western boundary of the Kruger National Park (KNP) in South Africa. Two hundred and eighty-three cattle from four communal dip tanks were longitudinally sampled after vaccination using an inactivated trivalent FMD vaccine (South African Territories (SAT) 1, SAT 2 and SAT 3). Blood samples were collected fortnightly over four months and antibodies were measured using a liquid-phase blocking ELISA. Young animals (<1 year old) had higher predicted baseline antibody levels that peaked by 14 days. Positive responses were transient and by 56 days post-vaccination antibody levels begun to decline below the serological threshold of 1.6 $\log_{10}$ titre. Predicted peak antibody levels only consistently reached 2.0 $\log_{10}$ titre for SAT 2 responses. Responses for SAT 2 tended to be longer, but in most cases the duration of antibody levels was short-lived. More research is necessary to determine the reasons for the limited duration of antibody responses, especially among younger cattle, in order to achieve more effective prophylactic vaccination.

**Keywords:** Cattle; Foot-and-mouth disease; Antibodies; Vaccine; Interface; Communal

## 1. Introduction

Foot-and-mouth disease (FMD) viruses are capable of infecting all cloven-hoofed species and in most cases causing an acute illness characterised by fever and lesions in the oral cavity, the skin-hoof junction of the coronary band, interdigital space and teats of lactating cows (Kitching, 2002; Arzt, et al., 2011). It is one of the world's most important animal pathogens because it causes losses in livestock trade and large-scale outbreaks (Paton, et al., 2010). Substantial funds are invested worldwide for prevention and control of FMD (OIE, 2012b).

FMD viruses (FMDV) exist as seven clinically indistinguishable serotypes, six of which are particularly important in Africa, *i.e.* A, O, C, South African Territories (SAT) types 1, 2 and 3 (Bachrach, 1968; Vosloo, et al., 2002; Knowles and Samuel, 2003; Rweyemamu, et al., 2008; Maree, et al., 2014; Tekleghiorghis, et al., 2014; Brito, et al., 2015). However, serotype C was last reported in Kenya in 2004. This serological classification was based on the inability of the viruses from different serotypes to induce cross-protection in animals (Pereira, 1976). Research has also demonstrated substantial antigenic variation within FMDV SAT serotypes (Vosloo et al., 1995; Vosloo et al., 1996; Bastos et al., 2003; Maree et al., 2011; Maree et al., 2015; Vosloo and Thomson, 2017) that may render available vaccines less effective.

There is evidence that currently available FMD vaccines against the SAT serotypes within the Southern African Development Community (SADC) region perform poorly and that this could be a reason for continued outbreaks in vaccinated populations of the region (SADC, 2010). Recent FMD outbreaks within the SADC region have increased in frequency and in most cases, these outbreaks have persisted for a longer time (FMD Bulletin, 2010; Penrith and Thomson, 2012; Jori et al., 2016). Overall, traditional FMD control measures have proven to be inadequate in some parts of the SADC during the last 10-15 years (FMD Bulletin, 2010; Thomson et al., 2013; Vosloo and Thomson, 2017). Tanzania, Botswana, South Africa,

Mozambique, Zambia, Zimbabwe, Namibia and Malawi have all reported outbreaks to the OIE/FAO Regional Laboratory Network during the past decade, with South Africa regaining its FMD Free status 2 years after the 2011 outbreaks within the free zone (Baipoledi et al., 2004; Jori et al., 2009; Dion et al., 2011; Brito et al., 2015; Vosloo and Thomson, 2017; OIE-WAHID, 2017).

FMD vaccine potency is determined based on the required dose to protect 50% of challenged animal ($PD_{50}$). Vaccines are classified as either "standard" or "high potency" (vaccine with >6 times the $PD_{50}$ antigen payload) based on the quantity of antigen (OIE, 2012a). A standard vaccine with a potency of more than $3PD_{50}$ and appropriate adjuvant is considered suitable for routine vaccination campaigns in FMD endemic locations (Elnekave, et al., 2013). FMD vaccines in endemic countries often contain antigens from more than one serotype, depending upon the epidemiological situation of the particular country. Cattle within the FMD Protection Zone with Vaccination of South Africa, along the western border of the Kruger National Park (KNP) and adjoining conservation areas, are routinely vaccinated against FMD every 4 months using a trivalent inactivated whole virus preparation containing SAT 1, SAT 2, and SAT 3 (DAFF 2014).

Serological assays, including virus neutralisation tests (VNT) and enzyme-linked immunosorbent assays (ELISA), can be 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 (Grant et al., 2016; Grant et al., 2017; McCullough et al., 2017). Despite the good correlation between serum antibody titres and protection, the presence or absence of substantial antibody titres are not always predictive of infection in individual animals after experimental challenge (Hunter, 1996; Cloete, et al., 2008; Sobrino, et al., 2001; Blignaut, et al., 2011; McCullough et al., 2017).

The objective of this study was to determine the level and duration of the antibody response induced by the current FMD vaccination programme in cattle within a communal farming area adjoining the KNP in South Africa.

## 2. Materials and methods

### 2.1 Ethical clearance

The study was approved by the Animal Ethics Committee (Project Number V010-12) of the University of Pretoria, Faculty of Veterinary Science and was performed according to national and international guidelines (South African National Standard, 2008). The work was also approved under Section 20 of the Animal Disease Act (Act 35 of 1984) of the Republic of South Africa: "Limitations on investigations, experiments and research with, and manufacture and evaluation of, certain products" (Application Number 12/11/1/1 Directorate: Animal Health, Department of Agriculture, Forestry and Fisheries). All samples collected were processed, packaged and transported to the laboratory according to the Regulations of the National Road Traffic Act, 1996 (Act No. 93 of 1996) of South Africa and under a veterinary red cross permit.

### 2.2 Study area

The study was conducted within the area under the authority of the Mnisi Tribal Council (the Mnisi Community), which is a communal farming area situated within the FMD Protection Zone with Vaccination, adjacent to provincial and private nature reserves contiguous with the KNP and falling within the Bushbuckridge Municipality, Mpumalanga Province. One of the major agricultural activities of the residents of this community is cattle farming, using an extensive free range system. Cattle in this area are routinely vaccinated against FMD virus free of charge by the Provincial Veterinary Service of Mpumalanga (MPVS) using a trivalent vaccine containing inactivated SAT 1, SAT 2 and SAT 3 viruses.

### 2.3 Sample size justification

The Mnisi Traditional Authority (MTA) has 15 communal dip tanks (livestock inspection points) with an estimated cattle population of 11,615 as at the time of the study (MPVS. Pers. Comm. May 2011). The sample size calculations were performed to estimate the proportion of cattle with liquid-phase blocking ELISA antibody titres of $\geq 1.6$ $\log_{10}$ at any sampling period post-vaccination. It was assumed that 80% of the study cattle would become seropositive at this level, and it was desired to estimate this proportion +/- 10% at the 95% level of confidence. A design effect of four was assumed to account for the clustering of cattle within herds and dip tanks. Based on these assumptions, the sample size was estimated as a minimum of 246 cattle.

### 2.4 Selection of cattle

Four cattle dip tanks in the Provincial Veterinary Service Animal Health Ward B1-B4 in the north-east of Bushbuckridge and under the Mnisi Traditional Authority, were purposely selected based on the scheduling of weekly cattle inspection for FMD (Fig. 1). This was due to practical considerations, i.e. to enable different locations to be sampled on four consecutive days. The four dip tanks were managed by two animal health technicians. Seven herds (groups of cattle owned by the same person) were conveniently selected at each communal dip tank after obtaining informed consent from the farmers. Ten cattle older than six months of age were purposely selected in the order of presentation from each participating herd. The age of selected cattle was determined based on dentition (Torell, et al., 2003) and available information from the herder, and subsequently categorised as <1 year, 1-2 years and >2 years of age. Selected cattle received designated ear-tags for identification.

### 2.5 Vaccination procedure

The Provincial Government's official Veterinary Services performed the routine cattle FMD mass vaccination programme during June 2012. The national and provincial veterinary authorities provide FMD vaccine free of charge within the FMD Protection Zone with

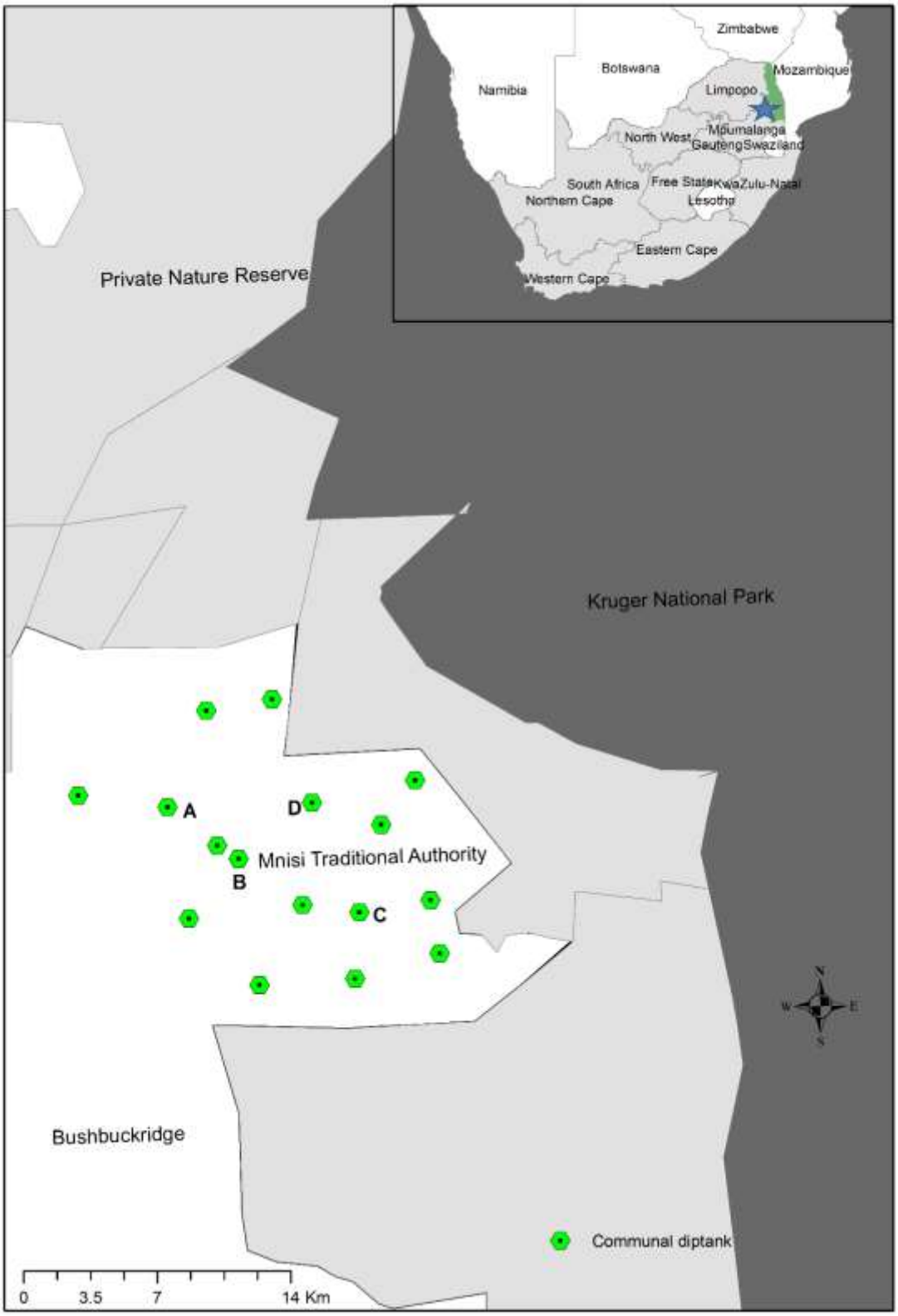


**Figure 1.** Map of the sampled locations for a foot-and-mouth disease virus vaccination study within the Mnisi Traditional Authority of Bushbuckridge, South Africa.

Vaccination. Regular FMD vaccination of cattle within the study area was biannually applied prior to 2008 using a previous vaccine product. The vaccination campaign was scheduled to be conducted every 4 months after introduction of the current product. Vaccines are distributed from a central cold store, where animal health technicians collect their allocation for administration within their local communities using procedures to maintain an effective cold-chain. Unfortunately, a 7-12 month inter-vaccination interval had lapsed since the previous FMD vaccination within the study area. During the study, cattle were vaccinated subcutaneously in the neck region using an automated syringe system. Each animal was injected with 5 ml of an aqueous aluminium hydroxide and saponin-adjuvanted inactivated trivalent FMD vaccine containing SAT 1, SAT 2 and SAT 3 vaccine strains (Aftovax®, Merial Animal Health Ltd/Botswana Vaccine Institute Gaborone). The vaccine batch number was 13309 with an expiry date of December 2012.

**2.6 Specimen collection**

Blood samples were collected on the day of vaccination (day 0) and on a further 8 occasions at two-week intervals during a four month follow-up period (0, 14, 28, 42, 56, 70, 84, 98, 112). 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 at the Hans Hoheisen Research Station within six hours of collection. Blood was centrifuged at 1450 g for 10 minutes immediately after delivery to the laboratory on the same day. Serum was decanted into sterile cryovials and stored at -20˚C and -80°C ultra-low temperature until testing.

**2.7 Laboratory testing**

Serum was stored frozen for 1-5 months depending upon the sampling round. Frozen sera were subsequently transported on ice from the Hans Hoheisen Research Station to the

Transboundary Animal Diseases Programme Laboratory of the Onderstepoort Veterinary Institute, Pretoria, for testing in batches. Samples were analysed for FMD-specific antibodies using a liquid phase blocking ELISA (LPBE) as previously described (Hamblin, et al. 1986). LPBE is a serotype-specific structural protein test for FMD antibodies. It is highly sensitive, provided that the virus or antigen used in the test is closely matched to the strain circulating in the field (OIE, 2012a). Assays were performed using an in-house ELISA for SAT 1, SAT 2 and SAT 3 viruses. This test is based on the serotype specific blocking of FMD heterologous antigen by antibodies in the test serum. Briefly, ELISA plates were coated with rabbit anti-FMD antibody. Sera premixed with FMD antigen was then added to the coated plates. If antibodies were present in the sera, they will block the antigen and prevent it from binding to the coating antibody. If there are no specific antibodies in the test sera, then the antigen will be available to be trapped on the plate and this is detected by a positive colour indicating negative test results. Antibody titres were expressed as the 50% end-point titres and sera with titres $\geq 1.6$ $\log_{10}$ were classified as seropositive (Cloete et al. 2008).

**2.8 Statistical analysis**

The Mann-Whitney U test was used to compare age between male and female cattle in the study population. Linear mixed models were fit to estimate the effect of covariates on $\log_{10}$ titres over time. Models included random effects for animal identification and evaluated fixed effect terms for natural log day, natural log day$^2$, natural log day$^3$ and sex. Independent models were fit for the nine combinations of FMDV serotype and cattle age categories. Predicted values for the fixed effects from the linear mixed model were used to generate plots of $\log_{10}$ titres over time. Descriptive data analysis for estimated marginal means and the linear mixed models were fit using IBM SPSS Statistics (Version 24, International Business Machines Corp., Armonk, New York, USA). Titre response curves for serotypes and age categorisation

were modelled using the ggplot2 package (Wickham, 2009) within R (R Core Team, 2017). Results were interpreted at the 5% level of significance.

## 3. Results

A total of 283 cattle were sampled at four communal cattle dip tanks during the 112 day study period. However, complete follow-up was not achieved for all selected cattle (Table 1; Supplementary Table 1). It was observed from the records in farmers' stock cards and the provincial veterinary services that an inter-vaccination interval of 7-12 months had lapsed since the previous FMD vaccination within the study area before the study. Sampled female cattle were older than males ($P<0.001$). Young animals (<1 year old) had higher predicted baseline antibody responses and reached peak levels by 14 days. However, responses were short-lived and by 56 days post-vaccination the predicted mean antibody levels had declined below the serological threshold of 1.6 $\log_{10}$ (Figure 2). Antibody peaks occurred at 28 days and persisted longer for animals aged >1 year. The duration of antibody responses was greatest for SAT 2 and least for SAT 3. Bulls >2 years old had significantly lower responses for SAT 1 and SAT 2 ($P = 0.034$ and $P = 0.005$, respectively). Sex differences were not observed for any other combination of FMDV serotype and age category. Predicted peak antibody responses only consistently reached 2.0 $\log_{10}$ titre for SAT 2 responses. Both older age groups (>1 year of age) appeared to have similar responses that were greater than younger animals (Table 2).

## 4. Discussion

The overall proportion of seropositive cattle at the time of first sampling (day 0) was less than 70% and likely due to a prolonged inter-vaccination interval. The standard vaccination frequency, as applied by the Provincial Veterinary Services, includes the primary vaccination (two doses, 2-8 weeks apart) followed by four-monthly booster vaccinations. However, the

**Table 1.** Number (%) of cattle sampled during the four month study period at the four study locations within the Mnisi Traditional Authority of Bushbuckridge, South Africa stratified based on location, age category, breed and sex.

| | Diptank | | | | Age | | | Breed | | | Sex | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | A | B | C | D | < 1 year | 1-2 years | >2 years | Brahman | Cross | Nguni | Female | Male | |
| 0 | 32 (42) | 45 (58) | 0 (0)* | 0 (0)* | 6 (8) | 15 (19) | 56 (73) | 26 (34) | 24 (31) | 27 (35) | 62 (81) | 15 (19) | 77 |
| 14 | 63 (28) | 47 (21) | 70 (31) | 45 (20) | 35 (16) | 33 (15) | 157 (70) | 24 (11) | 99 (44) | 102 (45) | 164 (73) | 61 (27) | 225 |
| 42 | 50 (23) | 51 (23) | 60 (26) | 57 (26) | 33 (15) | 33 (15) | 152 (70) | 20 (9) | 104 (48) | 94 (43) | 160 (73) | 58 (27) | 218 |
| 56 | 65 (36) | 0 (0) | 65 (36) | 53 (29) | 33 (18) | 23 (13) | 127 (69) | 21 (11) | 79 (43) | 83 (45) | 130 (71) | 53 (29) | 183 |
| 70 | 65 (30) | 38 (17) | 67 (31) | 49 (22) | 29 (13) | 30 (14) | 160 (73) | 26 (12) | 88 (40) | 105 (48) | 162 (74) | 57 (26) | 219 |
| 84 | 28 (39) | 44 (61) | 0 (0) | 0 (0) | 7 (10) | 16 (22) | 49 (68) | 14 (19) | 21 (29) | 37 (51) | 53 (74) | 19 (26) | 72 |
| 98 | 34 (17) | 48 (24) | 50 (25) | 66 (33) | 33 (17) | 29 (15) | 136 (69) | 21 (11) | 89 (45) | 88 (44) | 145 (73) | 53 (27) | 198 |
| 112 | 65 (27) | 54 (23) | 56 (23) | 64 (27) | 36 (15) | 38 (16) | 165 (69) | 31 (13) | 97 (41) | 111 (46) | 180 (75) | 59 (25) | 239 |
| Total | 402 (28) | 327 (22) | 368 (26) | 334 (23) | 245 (17) | 523 (18) | 1157 (81) | 213 (15) | 700 (49) | 742 (52) | 1212 (85) | 443 (31) | |

*Dip tanks C and D were vaccinated but not sampled on day 0

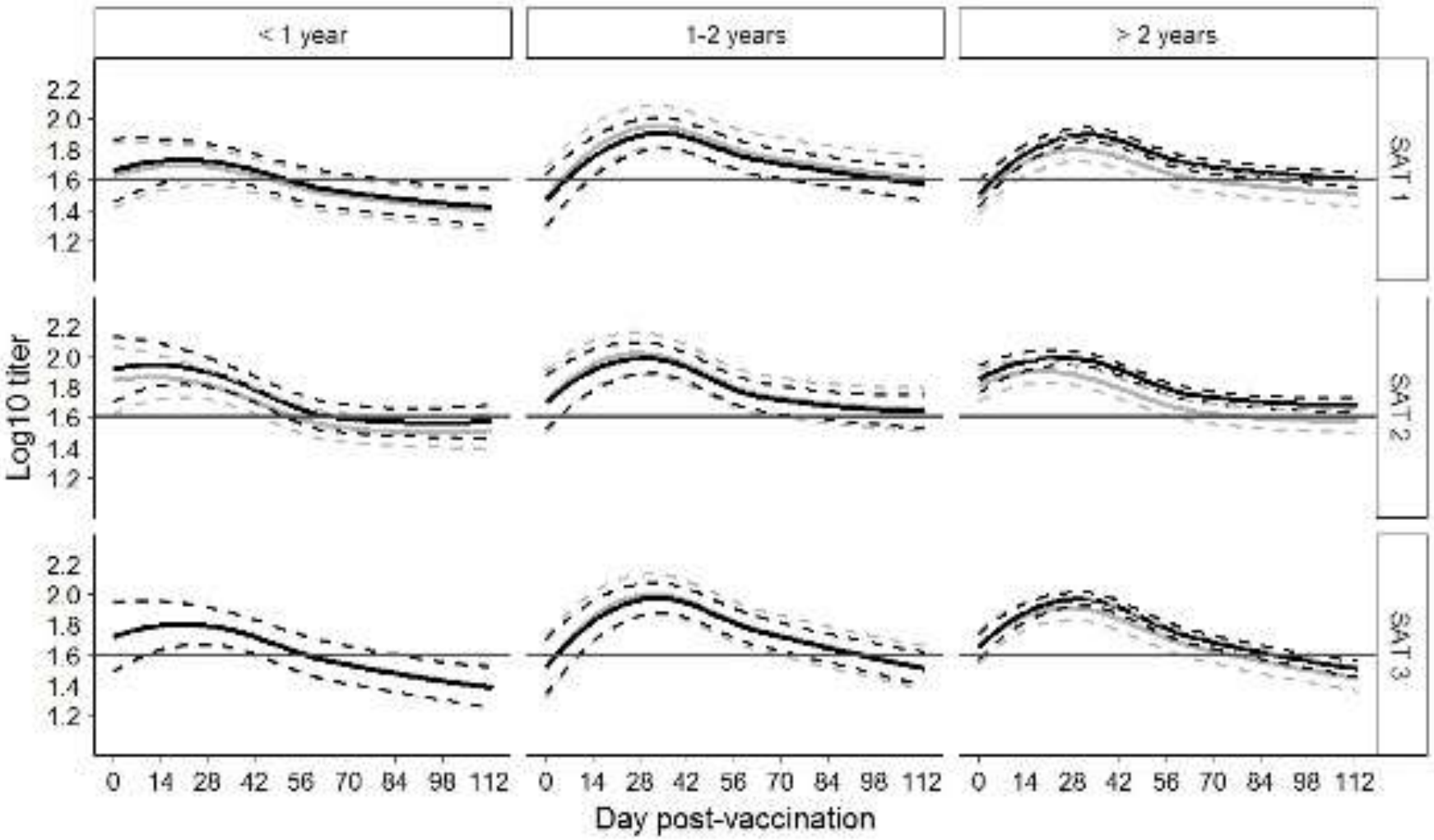


**Figure 2.** Predicted foot-and-mouth disease virus vaccination response curves by serotype and age categories for a 112 day study period in the Mnisi Traditional Authority of Bushbuckridge, South Africa. Responses from females are presented in black and male responses presented in gray. Solid lines correspond to predicted means and dashes are 95% confidence intervals.

**Table 2.** Estimated marginal means and peak titre for foot-and-mouth disease virus serotypes by age categories based on mixed effect linear models that included a random effect for animal in the Mnisi Traditional Authority of Bushbuckridge, South Africa.

| | Females | | Males | | |
|---|---|---|---|---|---|
| **Vaccination category** | **Peak titre (duration)** | **Mean titre (95% CI)** | **Peak titre (duration)** | **Mean titre (95% CI)** | **P value*** |
| **SAT 1** | | | | | |
| < 1 year | 1.80 (56) | 1.57 (1.47-1.68) | 1.77 (56) | 1.55 (1.44-1.66) | 0.739 |
| 1-2 years | 1.93 (112) | 1.72 (1.63-1.80) | 1.96 (>112) | 1.75 (1.61-1.88) | 0.693 |
| > 2 years | 1.96 (98) | 1.72 (1.68-1.76) | 1.87 (70) | 1.63 (1.55-1.71) | 0.034 |
| **SAT 2** | | | | | |
| < 1 year | 2.09 (70) | 1.72 (1.64-1.80) | 2.03 (56) | 1.65 (1.55-1.74) | 0.294 |
| 1-2 years | 2.15 (>112) | 1.79 (1.71-1.87) | 2.17 (>112) | 1.81 (1.67-1.94) | 0.849 |
| > 2 years | 2.16 (>112) | 1.82 (1.78-1.85) | 2.05 (84) | 1.72 (1.65-1.78) | 0.005 |
| **SAT 3** | | | | | |
| < 1 year | 1.87 (56) | 1.60 (1.48-1.72) | 1.88 (56) | 1.61 (1.48-1.74) | 0.930 |
| 1-2 years | 2.00 (98) | 1.74 (1.65-1.82) | 2.01 (98) | 1.75 (1.62-1.87) | 0.918 |
| > 2 years | 2.05 (98) | 1.74 (1.70-1.78) | 1.99 (70) | 1.68 (1.61-1.75) | 0.174 |

CI = confidence interval.

*Comparing titers between sexes (fixed effect term in the linear mixed model)

execution of immunisation programmes is dependent on the availability of vaccine and during outbreaks prophylactic vaccinations can be delayed as available vaccine is instead used for emergency vaccinations. In the present study, female cattle were older than sampled bulls. This is typical of production systems in sub-Saharan Africa as the cows are kept for breeding purposes. For this study, a cut-off titre of $\geq 1.6$ $\log_{10}$ was selected in order to describe the results. However, 2.0 $\log_{10}$ is indicative of good immunological protection afforded by vaccination as estimated by potency test results of different vaccine strains and animal species (OIE, 2012a). Younger animals had evidence of baseline antibody levels that was not similarly observed for the older animals. This might be a result of colostral antibodies from vaccinated dams or the unlikely situation of salient circulating field virus only affecting the youngest animals. Colostral antibodies have been known to interfere with vaccinal responses (Nicholls et al. 1984; Sadir et al., 1988; Patil et al., 2014; Elnekave et al., 2016b), and this might be the cause of the rapid decline in the antibody levels within these younger animals. In a related study in South America, calves with maternally derived antibodies (MDA) did not merely fail to respond to vaccination, but serum titres were depressed and only lasted for 60 days (Nicholls et al., 1984). In previous studies, young calves vaccinated against FMDV in the presence of MDA failed to have increases in the neutralising antibody titre when the applied vaccine incorporated aluminium and saponin adjuvants (Nicholls et al., 1984; Sadir et al., 1988; Patil et al., 2014). However, in other studies, the presence of MDA did not affect the neutralising antibody titre increase following vaccination (Elnekave et al., 2016b). An alternative explanation for the reduced response in the very young animals is the possibility that these animals never received the prescribed double primary dose of vaccine.

Peak antibody responses in this study occurred later in older animals and deviated from the expected rapid anamnestic response (7-10 days) often observed in vaccinated animals (Woolhouse, et al., 1996; Cloete, et al., 2008; Patil et al., 2014; Puentes et al., 2016). It could

be that the immune system in the older animals took longer to respond to the vaccine antigen due a sub-optimal nutritional status compared to younger animals. Body conditional scoring was not performed as part of this study and the inability to control for this factor is a limitation of the present study. No clinical signs of FMD were observed during the study period and no outbreaks were reported within South Africa during the study or immediately following its completion. The predicted response curves are consistent with a single vaccination without field virus exposure; however, the lack of confirmation using non-structural protein assays is a further limitation of the present study.

The duration of immunity is an important consideration for FMD vaccines (Hunter, 1998; Doel, 2003; Knight-Jones, et al., 2015). A vaccine that will induce a strong and sustained serological response is required for effective control (Cloete, et al., 2008; Parida, 2009). The conventional alhydrogel-saponin FMD vaccine in combination with the implementation of zoosanitary measures has been effective in eradicating FMD from European countries (Boldrini, 1978; Leforban and Gerbier, 2002; Kitching, 2005). However, vaccination has been ineffective in most parts of southern Africa. The alhydrogel-saponin vaccine presents a problem for large scale application under extensive conditions because the antibody response is short-lived and requires frequent application (Hunter, 1998; Cloete et al., 2008; Juleff et al., 2009; FAO, 2016; Vosloo and Thomson, 2017). Thus, cattle in endemic areas require revaccination at regular intervals of 4-6 months to ensure protective levels of antibodies (Cox, et al., 2003; Doel, 2003; Parida, 2009; OIE, 2012a; Vosloo and Thomson, 2017). In this study, vaccination did not elicit a sustained immune response beyond four months. This is consistent with findings from a study conducted in Mozambique in which only 10% of cattle vaccinated with a bivalent FMD vaccine (SAT 1 & SAT 2) were still seropositive four months after vaccination (Massicame, 2012). In a previous study in Kenya, FMDV serotype SAT 2 outbreaks persisted in dairy cattle herds despite revaccination at intervals of 4-6 months. The authors presumed that the reason for the

poor vaccine effectiveness was the antigen diversity of the SAT 2 serotype and a poor match between the field and vaccine strains (Lyons et al., 2015).

The SAT 2 antibody responses in this study were predicted to be better than the SAT 1 and SAT 3 responses. This might be attributed to a closer match between the vaccine virus and the ELISA antigen ensuring a stronger binding effect. Another possible explanation could be that the SAT 2 antigen is provided at a higher payload or is more thermo-stable relative to the SAT 1 and SAT 3 under prevailing field conditions. The SAT 2 viruses have been reported to have more sequence variation in the VP1 gene relative to other serotypes, for which it has been considered to be a broad antigenic variant (Maree et al., 2014; Lyons et al., 2015; Brito et al., 2016; Vosloo and Thomson, 2017). It might therefore be expected that vaccine and liquid-phase blocking ELISA antigen to be less likely to match relative to the other two serotypes.

Antibody responses appeared to vary by age with adult cattle (>2 years old) having higher antibody levels compared to younger cattle. However, there was little difference between heifers (1-2 years) and adults. This might be due to repeated exposures to the vaccine and subsequent anamnestic responses (Knight-Jones, et al., 2015; Elnekave, et al., 2016a). However, our results suggest that repeating vaccinations over and over again in older animals will not produce a substantially better response. The magnitude of the age effect might depend upon whether or not the cattle received the two initial primary doses as recommended by the local Veterinary Services. Consistent application of the two primary doses does not always occur due to inconsistent vaccine availability. Although unlikely, older cattle might also have been exposed to previous FMDV outbreaks within the protection zone.

Female cattle had higher titres compared to male cattle but the difference in the estimated titres did not appear to be clinically important. There is no biological explanation as to why female cattle would mount better serological responses, since vaccination occurs at the same time, and

all animals are managed within the same production system. However, it could be that adult bulls sometime receive an under-dose of vaccine antigen due to restraint and handling challenges at the dip tanks. The indigenous local Nguni breed accounted for the majority of the cattle population but breed did not have a significant effect on measured serological responses (data not presented).

This study has limitations including the fact that it was based on serological responses only and that information concerning the specific viruses in the vaccine was not available to the researchers. Therefore, the use of heterologous antigens in the liquid-phase blocking ELISA could have underestimated the proportion of cattle classified as seropositive. The use of liquid-phase blocking ELISA alone without virus neutralization testing as confirmation is another limitation to this study (Lavoria, et al., 2012). Sera were also not tested for FMD non-structural proteins, which would have provided better evidence that measured serological responses were only attributable to vaccination without concurrent exposure to a field virus.

Two of the study dip tanks were not sampled during the first week as a result of the on-going vaccination programme, which did not permit simultaneous sampling of cattle. Also, most farmers could not present their cattle for sampling at these same dip tanks at day 84 due to excessive rainfall. Some of the selected cattle were also not available for complete follow-up because farmers did not always present their entire herd due to the open and free-roaming grazing system.

The convenience sampling approach employed in this study might be a potential source of selection bias. The assumption of the random effect model is that the $\log_{10}$ titre values are normally distributed and this assumption appeared to be violated. The results of the models were consistent with the crude univariate results (data not presented); however, the impact of this assumption violation could not be measured directly.

## 5. Conclusions

The current cattle vaccination programme at the wildlife-livestock interface along the western border of KNP in the north-east Mpumalanga elicits adequate sero-conversion in a high proportion of vaccinated cattle. However, the predicted peak antibody response was often less than 2.0 $\log_{10}$ titre and the duration of the humoral response was relatively short-lived. More research is necessary to determine the reasons for the limited duration of vaccinal antibodies, especially within young animals, in effort to reduce the risk of FMD outbreaks in livestock at the wildlife-livestock interface of South Africa.

## 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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## Supplementary material

**Supplemental Table 1.** The number of samples collected from each enrolled cattle (follow-up) for a foot-and-mouth disease virus vaccination study comprising of eight sample periods over a 112 day duration in the Mnisi Traditional Authority of Bushbuckridge, South Africa.

| | Dip tank locations | | | |
|---|---|---|---|---|
| **Number of samples collected** | **A** **Number (%)** | **B** **Number (%)** | **C** **Number (%)** | **D** **Number (%)** |
| 1 | 2 (3) | 0 (0) | 1 (1) | 0 (0) |
| 2 | 1 (1) | 5 (7) | 1 (1) | 2 (3) |
| 3 | 3 (4) | 7 (10) | 3 (4) | 0 (0) |
| 4 | 4 (6) | 23 (34) | 3 (4) | 7 (10) |
| 5 | 17 (24) | 9 (13) | 12 (16) | 22 (31) |
| 6 | 18 (25) | 9 (13) | 18 (25) | 25 (35) |
| 7 | 7 (10) | 15 (22) | 35 (47) | 14 (20) |
| 8 | 20 (28) | 0 (0) | 0 (0) | 0 (0) |
| **Total** | **72 (25)** | **68 (24)** | **73 (25)** | **70 (26)** |

**Supplemental Table 2.** Number of animals enrolled (numerator) per herd relative to the total herd size (denominator) by dip tank location during the 112 day study period in the Mnisi Traditional Authority of Bushbuckridge, South Africa.

| | Dip tank | | | |
|---|---|---|---|---|
| **Herd number** | **A** | **B** | **C** | **D** |
| 1 | 11/13 | 10/12 | 10/19 | 10/37 |
| 2 | 7/7 | 10/39 | 11/13 | 10/22 |
| 3 | 13/20 | 10/40 | 10/36 | 10/19 |
| 4 | 11/11 | 7/8 | 10/11 | 10/15 |
| 5 | 10/61 | 11/13 | 11/23 | 10/18 |
| 6 | 10/25 | 10/17 | 10/45 | 10/17 |
| 7 | 10/12 | 10/21 | 11/23 | 10/43 |