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 livestock farming areas adjoining the Greater Kruger National Park Area within South Africa are part of the Foot-and-mouth disease (FMD) Protection Zone with Vaccination due to the proximity to wildlife reservoirs. FMD and its control affect the productivity of resource-poor farmers who often depend on livestock for their livelihoods. A cross-sectional study was performed with the objectives to evaluate the perceptions of farmers concerning FMD control, estimate the proportion of cattle with presumed protective antibody titres against FMD, as well as the proportion of herds with adequate herd immunity at the wildlife-livestock interface within Mpumalanga Province. One hundred and four farmers were interviewed with 73% (76/104) being cattle owners and the remainder hired cattle herders. The majority of respondents (79%, 82/104) reported a high level of satisfaction with the current animal health programmes in general. The educational level of the respondents varied by satisfaction level: the median (interquartile range; IQR) education level was standard 9 (2-12) for non-satisfied respondents, standard 3 (0-6) for little satisfied and standard 7 (2-11) for very satisfied respondents ($P = 0.036$). Animals are not always treated at FMD inspections points, but satisfied respondents were more likely to seek veterinary assistance ($P = 0.001$). The majority of respondents (92%, 96/104) identified the African buffalo (*Syncerus caffer*) as a risk factor for FMD outbreaks. Liquid-phase blocking ELISA antibody titres $\geq 1.6 \log_{10}$ were used to indicate positive serology secondary to FMD vaccination. At the time of sampling and relative to this threshold, 23% (95% confidence interval (CI): 12%-34%) of the sampled cattle had positive serology to SAT-1, 41% (95%CI: 33%-48%) to SAT-2 and 29% (95%CI: 19%-39%) to SAT-3. The median (IQR) time between the previous vaccination and sampling was 189 (168-241) days. The sampled cattle had a longer inter-vaccination interval as scheduled by state veterinary services and antibody levels were low at the time of the study. The majority of respondents expressed high satisfaction with the currently applied FMD vaccination programme, which provides an opportunity for progressive adaption of animal health programmes within the study area.

Keywords: Foot-and-mouth-disease; vaccination; control; wildlife interface; communal farming

1. Introduction

Foot-and-mouth disease (FMD) is an economically important disease of livestock in the tropics (Tanya, et al., 2003) and 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) and adjoining nature reserves (Greater KNP Area), due to the presence of African buffaloes (*Syncerus caffer*) and hence adjoining 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 virus 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 carry and maintain FMD virus and have been associated with outbreaks in impala (*Aepycerus melampus*) within the KNP and in cattle within the bordering communal farming areas (Vosloo, et al., 2009).

Resource-poor farmers frequently employ communal livestock production systems at interfaces with protected wildlife areas (Osofsky, 2005). The production outputs of these systems are often low because of husbandry practices, pasture quality and transmission of infectious diseases (Caron, et al., 2013). Communal farmers raise livestock to produce milk, meat, hides and manure that can be used to fertilise crops (Barrett, 1992; Chimonyo, et al, 1999; Dovie, et al., 2006). Cattle also provide draught power for the cultivation of crops and transportation of goods and services (Bayer, et al., 2004; Shackleton, et al., 2005). More importantly, cattle 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).

Disease control at the wildlife-livestock interface often employs vaccination and must consider issues related to vaccine delivery (Holden, et al., 1998; Heffernan and Misturelli, 2000) and characteristics of the affected farmers including perceptions and awareness of the affiliated technology (Bhattacharyya, et al., 1997; Bolorunduro, et al., 2004; Fandamu, et al., 2006; Homewood, et al., 2006). Important aspects related to the practicality of animal health interventions among the poor farming communities are access, affordability and acceptability (Heffernan and Misturelli, 2000). The overall goal of vaccination campaigns is a wide-scale adoption and establishment of protective immunity at the community, national and even regional levels (Mason and McGinnis, 1990; Humair, et al., 2002). Therefore these programmes must consider the perceptions of resource-poor farmers to ensure effective implementation (McLeod and Rushton, 2007; Heffernan, et al., 2008).

Cattle in the Protection Zone with Vaccination of South Africa, being at the interface with the wildlife of the Greater KNP Area, are scheduled to be vaccinated against FMD every four months using a trivalent inactivated vaccine containing vaccine antigens for all three SAT serotypes. The vaccinations are a governmental funded programme and carried out by the state veterinary service at no cost to the local farmers. Based on an assumed basic reproduction number of four for FMD, at least 75% of the cattle population should be immunised (vaccinated and developed sufficient neutralising antibodies) during vaccination campaigns to achieve herd immunity and prevent FMD virus epidemics (Woolhouse, et al., 1996). Chemically inactivated FMD vaccines induce short-lived antibody responses similar to other inactivated vaccines (Hunter, 1998; Maree et al., 2015). Therefore, vaccine manufacturers typically recommend that cattle in an endemic setting be revaccinated at least three times a year after an initial double primary course (Woolhouse, et al., 1996; Lubroth, et al., 2007), which is consistent with the four-monthly vaccination frequency as scheduled by the South African Veterinary Services in the Protection Zone with Vaccination within South Africa (DAFF, 2014).

The objectives of the present study were to evaluate the perceptions of farmers concerning FMD control and estimate the proportion of cattle with presumed protective antibody levels against SAT serotypes and thereby determine the prevalence of 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 from the University of Pretoria's Animal Ethics Committee at the Faculty of Veterinary Science (Project Number V010-12) and the Research Ethics Committee at the Faculty of Humanities (Project Number 2012-04-04). Act 35 (Animal Diseases Act) of 1984, Section 20 approval to perform research on a controlled disease was obtained from the Department of Agriculture, Forestry and Fisheries: Directorate of Animal Health (Application Number 12/11/1/1) of the Republic of South Africa. All samples collected were packaged according to the Regulations of the National Road Traffic Act, 1996 (Act No. 93 of 1996) of South Africa and transported under veterinary red-cross permits.

2.2 Study location and population

This study was conducted at the 15 provincial government livestock inspection points (dip tanks) within the land of the Mnisi traditional authority (Mnisi community), B1-B3 Animal Health Wards of the State Veterinary Office Bushbuckridge, located within the FMD

Protection Zone with Vaccination of the Mpumalanga Province in South Africa (Fig. 1). The land falling within the Mnisi community encompasses an area of 30,000 ha, and a population of 40,060 people living within 8,555 households. Available data suggest that 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 depend on land-based activities including cultivating home gardens, rearing livestock and gathering natural resources (Cousins, 1999; Shackleton, 2000; Dovie, et al., 2002).

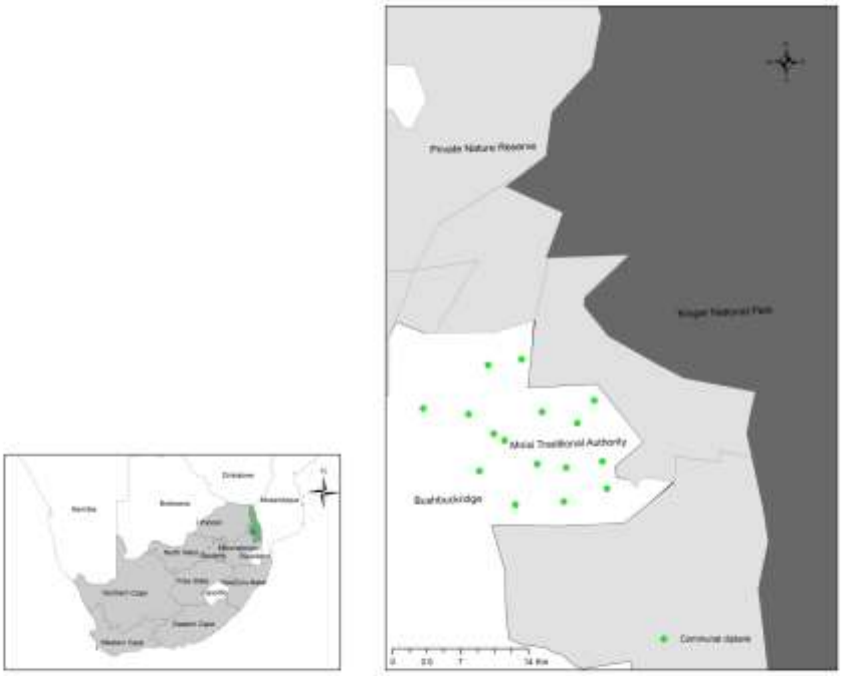

Figure 1. Map of the study area showing the distribution of the 15 communal dip tanks sampled

The Bushbuckridge area has generally sandy and infertile granite soils (Shackleton, 2000). Rainfall occurs mainly during summer months (October-April) and the total amount varies from 800 mm in the west to 500 mm per annum in the east (Shackleton, 2000). Increasing aridity moving eastward in the region is accompanied by escalating 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 also locally abundant but there are few donkeys and pigs. Two thirds of the boundary of the land of the Mnisi community forms an interface with provincial and private game reserves contiguous with the KNP and forming the Greater KNP Area. Cattle and wildlife are separated by game-proof veterinary control fences and the entire study region is situated within the FMD Protection Zone with Vaccination. Cattle in this communal farming area and FMD control zone are vaccinated thrice annually against FMD using a trivalent inactivated-vaccine (SAT-1, SAT-2 and SAT-3, Merial Animal Health Ltd/Botswana Vaccine Institute, Gaborone) at no cost to local farmers. Weekly animal disease inspections are conducted by the local veterinary services at the livestock inspection points aimed primarily at the detection of FMD. Inspections are performed on all cloven-hoofed livestock (cattle, sheep and goats) registered at the communal dip tanks, which is a statutory regulation by law from the Directorate of Animal Health (Animal Disease Act, 1984).

2.3 Study design and sample size justification

Cross-sectional studies employing a structured questionnaire administered through in-person interviews and clustered samplings of cattle by herds (group of cattle owned by the same person) were implemented. Sampling was conducted during May to June 2012 at the 15 communal cattle dip tanks within the area of the Mnisi community. The sample sizes were calculated to estimate the proportion of respondents with knowledge concerning FMD epidemiology and control and herd level proportion of cattle with FMD antibody titres in the absence of clinical outbreak, which was presumed to be vaccine induced. For the cross-sectional interviews, 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 respondents but was increased to 104 respondents to sample 10% of farmers at each communal dip tank (stratified sampling by dip tank, please Supplemental Table 1 for sampling fractions). However, clustering of respondents by cattle dip tank was not taken into account for calculating sample size.

For the cross-sectional cluster sampling of cattle by herds, the sample size was calculated to estimate the expected herd-level seropositivity (herd with ≥75% seropositive animals) with a 20% absolute error and at the 95% level of confidence. The sample size was calculated for an expected proportion of 50% since prior information was not available. The sample size was calculated as 24 herds; however, 30 herds were selected to allow for the enrolment of two herds per dip tank. Two herds (group of animals owned by a single farmer and living and grazing together) were conveniently selected at each dip tank using a pre-existing list of farmers from the cattle stock-registers kept by the local veterinary services and within each herd ten cattle (or the entire herd when <10) were selected. Sera with liquid-phase blocking ELISA (LPBE) antibody titres $\geq 1.6 \log_{10}$ were classified as seroconversion secondary to vaccination (Cloete, et al., 2008). Vaccination is performed at dip tanks by animal health technicians during scheduled FMD inspection times and it was therefore expected that within dip tank variability would be less than among dip tank variability. A uniform number of herds per dip tank were therefore selected and clustering within dip tanks was not considered for sample size estimation.

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, length 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 also 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 (yes-favourable, no-unfavourable), vaccination can make cattle sick (yes-unfavourable, no-favourable), vaccination can cause abortion in cattle (yes-unfavourable, no-favourable), vaccination improves cattle wellbeing (yes-favourable, no-unfavourable), vaccination can reduce feed intake in cattle (yes-unfavourable, no-favourable), sick cattle should be presented for vaccination (yes-unfavourable, no-favourable), and pregnant cattle should be presented for vaccination (yes-favourable, no-unfavourable). The complete questionnaire is available as supplemental material.

Questionnaires were administered through an in-person interview in the local language (Xitsonga) after translation from English. Within each communal dip tank, 10% of the registered livestock owners/herders were conveniently selected as they presented their cattle for inspection. The study was conducted during a period that coincided with a routine FMD 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 official owner-stock cards and veterinary services livestock registers at the time of questionnaire administration. Official veterinary reports were retrospectively reviewed to confirm data concerning FMD vaccination and dip tank attendance.

2.5 Cattle sampling and testing

Farmers were conveniently selected as they presented their cattle for regular weekly FMD inspection at the communal dip tanks. At least ten eligible cattle were selected in each herd based on their order of presentation. The age of selected animals was determined by dentition and available information from farmers and herders. Blood samples were collected from the mid-coccygeal or jugular vein into plain 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 transported on ice to the Transboundary Animal Disease Programme (TADP) Laboratory of the Onderstepoort Veterinary Institute, Pretoria, for testing. Serum samples were tested for antibodies against FMDV structural proteins (indicative of vaccination or exposure to field viruses) using liquid-phase blocking ELISA (Hamblin, et al., 1986) employing TADP-developed reagents for SAT-1, SAT-2 and SAT-3. Sera were categorised as seropositive at a liquid-phase blocking ELISA titre cut off of $\geq 1.6 \log_{10}$ for each serotype as reported previously (Cloete et al., 2008). Herds were classified as having adequate herd immunity if at least 75% of sampled cattle had titres greater than this threshold.

2.6 Data analysis

Categorical data were described using percentages and 95% confidence intervals (CI) and continuous data were described using medians and interquartile ranges (IQR). Chi-square and Fisher exact tests were used to compare proportions across categorical variables and Kruskal-Wallis tests were used to compare quantitative data. Significance was set as P<0.05. Descriptive data analysis was performed with EpInfo^{TM} (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). 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 sizes of cattle at each communal dip tank. Seroprevalences were estimated using dip tank as a stratifying variable, herd as a clustering variable, herd size as the weighting variable, and a finite population correction for variability estimates.

3. Results

3.1 Questionnaire

One hundred and four respondents participated in the study, with the majority of respondents being cattle owners (Table 1.). The median age of respondents was 48 (IQR: 33-66) years. Twenty-one percent of the respondents had no formal education qualification, 38% had completed primary education, and 36% completed secondary education. The median (IQR) number of cattle owned by respondents was 11 (6-19) heads of cattle. The median time involved in livestock farming was 13.5 (6-73) years. Married respondents had a median herd

Table 1. The association between levels of satisfaction with dip tank activities and potential categorical predictors in 104 livestock farmers sampled within the FMD Protection Zone with Vaccination in South Africa during 2012.

		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	11 (5-19)	21	28 (19-39)	47	62 (51-72)	0.457
Hired handlers	28	2	7 (2-23)	5	18 (8-36)	21	75 (57-87)	
Gender								
Male	87	9	10 (6-19)	21	24 (16-34)	57	66 (55-75)	0.797
Female	17	1	6 (1-27)	5	29 (13-53)	11	65 (41-83)	
Marital status								
Single	27	3	11 (4-28)	5	19 (8-37)	19	70 (52-84)	0.615
Married	57	6	11 (5-21)	15	26 (17-39)	36	63 (50-74)	
Divorced/Widowed	21	1	5 (1-23)	8	38 (21-59)	12	57 (37-76)	
Most important source of income								
Livestock	103	9	9 (5-16)	26	25 (18-34)	68	66 (54-74)	0.097
Crop	11	0	0 (0-26)	6	55 (28-79)	5	45 (21-72)	
Other animals kept								
Pig	13	1	8 (1-33)	6	46 (23-71)	6	46 (23-71)	0.710
Goat	40	3	8 (3-20)	11	27 (16-43)	26	65 (50-78)	
Chicken	78	6	8 (4-16)	21	27 (18-38)	51	27 (18-38)	
Source of drinking water								
Pipe	3	0	0 (0-56)	2	67 (21-94)	1	33 (6-79)	0.510
Well	85	8	9 (5-17)	20	24 (16-34)	57	67 (57-76)	
Pond	19	2	11 (3-31)	6	32 (15-54)	11	58 (36-77)	
Disease management practices								
Contacting a veterinarian	100	7	7 (3-14)	26	26 (18-35)	67	67 (57-75)	0.001
Self-treatment	12	5	42 (19-68)	3	25 (9-53)	4	33 (14-61)	
Grazing management								
Contact with wildlife	20	1	5 (1-24)	4	20 (8-42)	15	75 (53-89)	0.907
Grazing adjacent to the Park	53	3	6 (2-15)	13	25 (15-38)	37	70 (56-80)	

CI = confidence interval. FMD = foot-and-mouth disease *Based on chi-square tests comparing satisfaction levels.

size of 8 (5-15) versus 9.5 (6-15) for the other categories combined (P = 0.418). The median frequency of FMD vaccination reported by respondents was 2 (2-3) times per year. (Regular FMD cattle vaccinations within the study area were bi-annually applied prior to 2008 using a previous vaccine product).

All respondents indicated livestock farming as their major source of income in addition to crop based farming (Table 1). Animals such as pigs, goats and chickens were reared among respondents. Respondents indicated the use of piped water, well and open ponds as a source of water to their livestock, with the majority using well water (79%).

Majority of the respondents indicated dipping against ticks and other ectoparasites as their most favourite dip tank activities. Ninety-six percent of respondents reported that they called for veterinary assistance whenever there is a problem in their herds, while few indicated self-treatment as an option in addition to requesting veterinary assistance (Table 1). The level of satisfaction varied between farmers that requested veterinary assistance (P = 0.001).

Buffalo escape from the nature reserves and KNP was perceived to be the highest risk for FMD outbreaks among respondents, followed by the introduction of new animals and grazing adjacent to the fences of the nature conservation areas. A number of the respondents indicated knowledge of FMD as a disease that can cause lesions on the tongue, feet and udder/teats of lactating cows (Table 1). The average daily grazing distance among respondents was variable with the majority of respondents reporting a daily average trekking distance of 1-10 km from where they lived.

Seventy-nine percent of the respondents were very satisfied with the current vaccination programme, while 5% were not satisfied at all. The median education level of respondents varied over levels of satisfaction (P = 0.036) and was standard 9 (IQR: 2-12) for non-satisfied respondents, standard 3 (0-6) for the little satisfied respondents and standard 7 (2-11) for very

Table 2. The association between the levels of satisfaction with dip tank activities and potential continuous predictors in 104 livestock farmers sampled within the FMD Protection Zone with Vaccination in South Africa during 2012.

	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 comparing variables among the three satisfaction levels

Table 3. Frequency of responses to questions that could affect farmers' participation in vaccination programs as determined for 104 livestock farmers sampled within the FMD Protection Zone with Vaccination in South Africa during 2012.

	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		

satisfied respondents (Table 2), with standard 1-6, being primary school level and Standard 7-12 being high school level respectively.

The majority of respondents had favourable perceptions to FMD vaccination (Table 3); however, some believed that pregnant animals should not be presented for vaccination. Using the Kruskal-Wallis tests, the vaccination perception score of respondents varied over level of satisfaction with 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, originating from two herds at each of the 15 communal dip tanks within the study area. The median (IQR) age for animals sampled was 4.5 (2.5-6.0) years and the median (IQR) period since their last FMD vaccination was 189 (168-241) days. Relative to a LPBE antibody titre of $\geq 1.6 \log_{10}$, seroprevalences after adjusting for clustering and sampling fractions were 23% (95%CI: 12-34), 41% (95%CI: 33-48) and 29% (95%CI: 19-39) to SAT 1-3, respectively. Median titres for each SAT serotype descriptively varied among herds and dip tanks (Table 4), with the SAT-2 antibody titres appearing to be more consistent relative to SAT-1 and SAT-3 in the sampled herds. Seropositivity was less than 75% for all SAT serotypes in all but a single herd. One herd had a marked serological response for SAT-3 with 80% seropositivity. Fifty percent and 60% of cattle sampled from two other herds were also seropositive for SAT-3 virus.

Eighteen percent (95%CI: 10-29) of male and 21% (95%CI: 16-27) of female cattle were seropositive for SAT-1 (Table 5; P = 0.575). Seropositivity was highest in animals older than

Table 4. Proportion of seropositive cattle with median (IQR) Log_{10} antibody titre by herds in 286 cattle sampled from 30 herds within the FMD Protection Zone with Vaccination in South Africa during 2012.

		SAT-1		SAT-2		SAT-3	
Herd	**(n)**	**Seropositive %* (95%CI)**	**Median log_{10} (IQR)**	**Seropositive %* (95%CI)**	**Median log_{10} (IQR)**	**Seropositive %* (95%CI)**	**Median log_{10} (IQR)**
A1	(10)	0 (0-26)	<1.30 (<1.30-<1.30)	30 (8-62)	<1.30 (<1.30-1.65)	0 (0-26)	<1.30 (<1.30-<1.30)
A2	(10)	40 (14-71)	1.35 (<1.30-1.60)	70 (38-92)	1.65 (1.28-2.10)	0 (0-26)	<1.30 (<1.30-1.23)
B1	(10)	50 (21-79)	1.60 (<1.30-2.03)	60 (29-86)	1.65 (1.30-2.03)	50 (21-79)	1.40 (<1.30-1.93)
B2	(10)	50 (21-79)	1.55 (<1.30-1.80)	60 (29-86)	1.65 (1.50-2.03)	80 (48-97)	1.65 (1.50->2.20)
C1	(10)	50 (21-79)	1.65 (<1.30-1.83)	50 (21-79)	1.70 (1.45-2.03)	60 (29-86)	1.60 (<1.30-1.70)
C2	(10)	0 (0-26)	<1.30 (<1.30-1.23)	40 (14-71)	1.35 (<1.30-1.60)	30 (8-62)	1.35 (<1.30-1.60)
D1	(7)	0 (0-35)	<1.30 (<1.30-<1.30)	29 (5-67)	1.40 (<1.30-1.70)	14 (1-53)	<1.30 (<1.30-<1.30)
D2	(10)	30 (8-62)	<1.30 (<1.30-1.60)	30 (8-62)	1.35 (<1.30-1.65)	30 (8-62)	<1.30 (<1.30->2.20)
E1	(10)	30 (8-62)	1.30 (<1.30-1.73)	50 (21-79)	1.55 (1.28-1.95)	30 (8-62)	<1.30 (<1.30->2.20)
E2	(10)	10 (1-40)	<1.30 (<1.30-1.28)	30 (8-62)	1.35 (<1.30-1.70)	10 (1-40)	<1.30 (<1.30-<1.30)
F1	(10)	10 (1-40)	<1.30 (<1.30-<1.30)	10 (1-40)	<1.30 (<1.30-1.25)	10 (1-40)	<1.30 (<1.30-<1.30)
F2	(10)	10 (1-40)	<1.30 (<1.30-<1.30)	10 (1-40)	<1.30 (<1.30-1.28)	10 (1-40)	<1.30 (<1.30-<1.30)
G1	(10)	20 (4-52)	<1.30 (<1.30-1.30)	30 (8-62)	<1.30 (<1.30-1.63)	20 (4-52)	<1.30 (<1.30-1.38)
G2	(7)	29 (5-67)	1.40 (<1.30-1.80)	43 (12-78)	1.30 (<1.30-2.00)	29 (5-67)	1.50 (<1.30-1.45)

H1 (10)	40 (14-71)	1.35 (<1.30-1.70)	60 (29-86)	1.70 (1.40-2.00)	40 (14-71)	1.40 (<1.30-1.63)
H2 (10)	20 (4-52)	<1.30 (<1.30-1.30)	30 (8-62)	1.50 (<1.30-1.78)	20 (4-52)	<1.30 (<1.30-1.35)
I1 (10)	0 (0-26)	<1.30 (<1.30-<1.30)	50 (21-79)	1.60 (<1.30-1.70)	0 (0-26)	<1.30 (<1.30-1.25)
I2 (6)	17 (1-59)	<1.30 (<1.30-1.35)	17 (1-59)	<1.30 (<1.30-1.35)	17 (1-59)	<1.30 (<1.30-1.35)
J1 (10)	30 (8-62)	<1.30 (<1.30-1.73)	30 (8-62)	<1.30 (<1.30-1.60)	20 (4-52)	<1.30 (<1.30-1.30)
J2 (10)	20 (4-52)	<1.30 (<1.30-1.33)	20 (4-52)	1.35 (<1.30-1.58)	20 (4-52)	<1.30 (<1.30-1.33)
K1 (8)	13 (1-48)	<1.30 (<1.30-<1.30)	25 (4-61)	1.25 (<1.30-1.80)	25 (4-61)	<1.30 (<1.30-1.50)
K2 (10)	20 (4-52)	<1.30 (<1.30-1.30)	60 (29-86)	1.65 (<1.30-1.73)	20 (4-52)	<1.30 (<1.30-1.53)
L1 (10)	30 (8-62)	<1.30 (<1.30-1.78)	30 (8-62)	1.50 (<1.30-1.75)	30 (8-62)	<1.30 (<1.30-1.78)
L2 (10)	20 (4-52)	<1.30 (<1.30-1.45)	40 (14-71)	1.50 (<1.30-1.70)	40 (14-71)	1.35 (<1.30-1.80)
M1 (9)	0 (0-28)	<1.30 (<1.30-1.45)	56 (24-84)	1.60 (<1.30-1.70)	0 (0-28)	<1.30 (<1.30-1.30)
M2 (10)	0 (0-26)	<1.30 (<1.30-<1.30)	40 (14-71)	1.30 (<1.30-1.65)	10 (1-40)	<1.30 (<1.30-1.25)
N1 (9)	11 (1-44)	<1.30 (<1.30-<1.30)	0 (0-28)	<1.30 (<1.30-<1.30)	33 (9-67)	1.40 (<1.30-1.60)
N2 (10)	20 (4-52)	1.30 (<1.30-1.58)	30 (8-62)	<1.30 (<1.30-1.85)	20 (4-52)	<1.30 (<1.30-1.58)
O1 (10)	0 (0-26)	<1.30 (<1.30-<1.30)	60 (29-86)	1.60 (<1.30-1.93)	10 (1-40)	<1.30 (<1.30-1.25)
O2 (10)	30 (8-62)	<1.30 (<1.30-1.83)	40 (14-71)	<1.30 (<1.30-1.88)	30 (8-62)	<1.30 (<1.30-1.75)

*Based on ≥1.6 $\log_{10}$ titre value.
CI = Confidence interval

2 years, 22% (95%CI: 17-28), although age was not a significant predictor of SAT-1 serological status (P = 0.125). Brahman cattle had lower SAT-1 seropositivity 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, with none being significant.

Table 5. Serological responses to SAT-1, SAT-2 and SAT-3 on the basis of sex, age and breed (titre ≥1.6 Log_{10}) in 286 cattle sampled within the FMD Protection Zone with Vaccination in South Africa during 2012.

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

A retrospective review of the records of the previous mass vaccination campaign across the study area indicated high vaccination coverage of over 90% at the dip tank level.

3.3 Representativeness of sample

The average herd sizes of sample farmers were larger than the population average for 11 of the sampled dip tanks (Supplemental Table 1). However, retrospective analysis of dip tank records revealed excellent attendance of farmers at the scheduled FMD inspections during the study period (Supplemental Table 2)

4. Discussion

The primary aim of this study was to evaluate the current perceptions of communal livestock farmers concerning FMD epidemiology, vaccination and control. A second objective was to determine the proportion of cattle with presumed protective antibody levels against FMD and the proportion of herds with adequate herd immunity. To our knowledge, this is the first survey regarding the perceptions of communal farmers concerning FMD vaccination since the establishment of the Great Limpopo Transfrontier Conservation Area (GLTFCA).

More respondents (76/104) were involved in herding their own cattle rather than employing paid herders. Both men and women were involved in herding animals in this area with men accounting for 84% 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 (payment 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. This might be due to a common belief among farmers that vaccination can adversely affect neonatal/ foetal development, even though the employed FMD vaccine is inactivated and not contra-indicated in pregnant animals. The apparent disinclination of having pregnant animals vaccinated by the majority of respondents did not show any notable effect on the achieved output of immunisations. However, sickness was not perceived as a reason to avoid vaccinating animals. No other evaluated factors were perceived to affect farmers' presentation of cattle for vaccination. Cattle are important to communal farmers for special ceremonial gatherings including weddings, funerals and circumcision (Bayer, et al., 2004) suggesting that married households might be expected to have larger herd sizes. Although not statistically significant, married households actually had smaller herd sizes when compared to other categories.

Seventy-four percent of the respondents had either a primary or secondary education qualification, indicating that the majority of farmers were literate and therefore more likely to adopt innovations (Fandamu, et al., 2006). The majority of respondents (79%) were satisfied with the vaccination programme and this may be due to no FMD outbreaks being detected within the study area from 1979 up until the time of this study. However, the majority of non-satisfied respondents had higher education qualifications. This suggests that more educated farmers perceived inadequacies in the existing animal health programmes or their implementation.

FMD is considered the most important livestock disease at the wildlife-livestock interface in southern Africa (Vosloo, et al., 2002a; Vosloo, et al., 2002b; 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 that causes 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 (which includes regular weekly inspection of susceptible cattle and monthly inspection of other cloven-hoofed livestock), few farmers themselves seem to have adequate knowledge to identify FMD. Therefore, there appears to be a need to strengthen educational programmes concerning FMD and other important livestock diseases among communal farmers. The high number of respondents (96%) indicating that they request veterinary assistance for disease situations is a reflection of the animal health awareness within the study area. Veterinarians, in addition to the para-veterinarians (animal health technicians) working for the state veterinary services, could therefore be an important source of educational material for farmers in the area. However, this area is not representative of the entire Bushbuckridge region due to the presence of a University of Pretoria animal health clinic and routine dip tank visits by veterinary students, in addition to the regular presence of animal health technicians.

With the global increases in the human population, there is a need to improve livestock production across the entire livestock industry. During the last decades, the demand for meat and milk has increased globally, particularly in developing countries. Beef is also in high demand for export markets (Delgado, et al., 1999; Delgado and Narrod, 2002; 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 the study area 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 propagation of FMD outbreaks. Respondents indicated that wildlife would increase the risk for disease in livestock when they shared water points and grazing. Furthermore, the African buffalo was reported by the majority of respondents (92%) as representing a risk for disease transmission to cattle. Contacts between livestock and wildlife have been previously reported to occur at the interface (Brahmbhatt, et al., 2012); however, interactions are limited due to the physical separation by veterinary control game fences. These findings indicate that some knowledge concerning FMD epidemiology has been transferred to the local community.

Cattle in the Protection Zone with Vaccination, and thus also the study area, are routinely vaccinated against FMD using an inactivated trivalent-vaccine containing SAT-1, SAT-2 and SAT-3 vaccine strains. However, the proportion of cattle with high levels of detectable antibody against these FMD serotypes was low, suggesting that the area could have been at risk for the active spread of FMD virus at the time of the study. This finding is consistent with a previous study where the antibody level induced by alhydrogel-saponin SAT type vaccine preparation fell below the 1.6 $\log_{10}$ virus neutralisation levels between two and three 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 11 months post-vaccination (Hunter, 1998; Cloete, et al., 2008). In addition to the properties of the vaccine antigen and the adjuvant used, other factors also influence the duration of protective antibody levels. These include matching the vaccine antigen to the locally prevailing field viruses and the condition and nutritional state of the animal. These factors might also have been a cause of the low proportion of cattle with high levels of antibodies. This low proportion at the time of the study, despite overall high vaccination coverage, implies that further improvements are necessary. Therefore, the high satisfaction level of respondents might not be a true indication of the effectiveness of the control programme.

In this study, heterologous antigens were used in the liquid-phase blocking ELISA because information concerning the viruses included in the commercial vaccine is proprietary. This might be an explanation for the high variability in measured antibody levels observed for SAT-1 and SAT-3 at the time of sampling. The manufacturer recommends administering two doses one month apart when cattle are first vaccinated, which is also included in the routine FMD vaccination programme. However, the application of the double primary immunisation might not be feasibly applied consistently due to emergency vaccinations during concurrent outbreaks in other areas.

SAT-2 antibody levels appeared to be more consistent relative to the SAT-1 and SAT-3, and this might suggest a closer antigenic relationship between the vaccine strain and the test antigens. This could also explain why more herds had seropositive proportions greater than 50% for SAT-2 antibodies compared to the other serotypes. The SAT-2 viruses have been reported to have more sequence variation in the VP1 coding region relative to other serotypes and vaccine manufacturers often select immunodominant vaccine strains with broad antigenic coverage (Paton, et al., 2005; Paton, et al., 2011; Maree et al., 2015). The antibody response to SAT-3 viruses was relatively poor in adult cattle and demonstrated high variability between herds and dip tanks (similar to SAT-1). However, there were three herds with greater seropositivity (50% – 80%). No clinical signs of FMD were evident during the weekly inspections by trained animal health technician. However, this might represent exposure to, and possible circulation of, SAT-3 viruses in isolated herds within the study area, which has been suggested previously (Jori, et al., 2014). Other FMD serotypes have also been reported to cause subclinical disease in cattle. In 2011, there was a large outbreak of SAT-1 in South Africa (KwaZulu-Natal), outside the FMD Protection Zone with Vaccination, which was reported to the OIE as being completely subclinical (OIE, 2011).

Age was not a significant predictor for seropositivity, contrary to our expectation that older cattle would have larger seropositive proportions because of exposure to repeated immunisations. This could be due to rapid antibody decline or poor stimulation of memory B-cells. Sex was also not a significant predictor for any of the three SAT type FMD viruses. However, the Nguni breed descriptively had higher seropositive proportions suggesting that the local breed might have better immune responses to vaccination. However, this requires further investigation employing larger sample sizes.

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 viruses (Ahl, et al., 1983; Sutmöller, et al., 1983; Pay and Hingley, 1987). Therefore serological evidence of FMD antibodies in vaccinated cattle in the absence of circulating field viruses can be used as an indicator of protection. The low proportion of seropositivity observed might be an indication of rapidly declining humoral response and a reduced level of protection.

The employed data analysis did not include multivariable ordinal regression analyses to investigate the joint effects of questionnaire data on reported satisfaction levels. Other limitations include the fact that information was obtained from livestock owners and herders using an in-person interview concerning FMD vaccination and control. The questionnaire was not pre-tested prior to administration and respondents might have understood some questions. Also, respondents might not have offered their true opinions in the interview because of the presence of government animal health technicians. Selection bias is also a potential problem because of the convenient selection of participants for the interview, as farmers who avail themselves early for the dipping might be the more enlightened respondents. Potentially, the better farmers might have participated in the study as evidenced by the larger average herd sizes. However, the study area had excellent dip tank attendance suggesting that all farmers in the region were eligible to participate and those that did not were unlikely to have different opinions concerning disease control activities. Future studies should include the views of all stakeholders (veterinarians and other animal health care workers) involved in vaccine administration. Cattle were also selected based on the convenient sampling of farmers and therefore might not be an accurate representation of the target population. It is theoretically possible that herds with adequate antibody levels were not tested due to the convenient sampling approach. The incomplete sampling of cattle within the herd is another limitation that might affect 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 true proportion of seropositive cattle.

The median time period between sampling and the most recent FMD vaccination was 189 days. This interval was 69 days longer than the 4-month interval that has been scheduled by the veterinary services since 2008. This delay occurred due to the need for emergency vaccination because of FMD outbreaks in other areas within the FMD Protection Zone with Vaccination. In August 2013, a SAT-2 FMD outbreak occurred in cattle within the study region that was immediately diagnosed in its acute phase by the state veterinary services.

5. Conclusions

The majority of respondents were literate and therefore more likely to adopt innovations. Respondents had some knowledge related to FMD epidemiology as the highest perceived risk for outbreaks was buffaloes escaping from the nature conservation areas followed by the introduction of new stock to their herds. However, few respondents recognized lesions consistent with FMD when described in the local language. More education is therefore required considering the importance of FMD at the interface. Overall, seropositivity was less than 75% for all the SAT serotypes, except for SAT-3 in one herd, and this could represent a risk for FMD outbreaks to occur within the study area. The overall positive perceptions of livestock farmers towards the animal health programmes highlights the strength of the local veterinary service delivery and suggests that progressive adaption of disease control programmes could be possible.

Conflict of interest

None

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