Efficacy of a foot-and-mouth disease vaccine against a heterologous SAT1 virus challenge in goats

D D Lazarus[1,2,*], F Peta[3], D Blight[1,3], J Van Heerden[3], P B Mutowembwa[3], L Heath[3], B Blignaut[1], P A Opperman[1,3], G T Fosgate[1]

[1]University of Pretoria, Faculty of Veterinary Science, Department of Production Animal Studies, Onderstepoort, South Africa.

[2]National Veterinary Research Institute, Foot-and-Mouth Disease Virus Research Laboratory, Vom, Nigeria.

[3]Agricultural Research Council, Onderstepoort Veterinary Research, Transboundary Animal Diseases, Onderstepoort, South Africa.

*Email: lazdav2003@yahoo.co.uk dlazarus@nvri.gov.ng (D.D. Lazarus)

Tel: +27 74 634 7529

Highlights

- Domestic goats are important FMD susceptible host species.
- However, their role in the epidemiology of the disease is poorly understood.
- A high-potency FMD vaccine induced clinical protection in goats against a heterologous challenge with FMDV SAT1.

Abstract

Goats are susceptible to infection with foot-and-mouth disease virus (FMDV), but their role in the epidemiology of the disease and response to vaccination is poorly understood. In southern Africa, FMDV serotypes Southern African Territories (SAT) 1, 2 and 3 are known to be endemic. In this study, we evaluated the efficacy of a pentavalent FMD vaccine in goats against heterologous challenge with a pool of field SAT1 FMDV. Forty FMD sero-negative goats (6-12 months of age) of mixed sexes were randomly allocated to one of five treatment groups: full cattle dose (2 ml), 1/3rd (0.67 ml), 1/6th (0.33 ml), 1/12th (0.16 ml) or unvaccinated placebo control. Goats were vaccinated with an inactivated pentavalent FMD vaccine containing serotypes SAT1, SAT2 and SAT3 on day 0 and revaccinated at day 20 post vaccination. Thereafter, thirty-four goats were challenged by tongue inoculation at day 41 post-vaccination using $10^{4.57}$ 50% tissue culture infective dose ($TCID_{50}$) FMDV SAT1 pool. Animals were examined daily and clinical signs were scored. Rectal temperatures were measured daily, with temperatures ≥40°C defined as fever. Clinical specimens (nasal, oral and rectal swabs) were collected on days 0, 2, 4 and 6 post challenge. Viral shedding was determined using reverse-transcriptase real-time PCR. None of the goats vaccinated with the full cattle dose developed secondary lesions. All vaccinated groups had lower temperatures compared to the unvaccinated controls ($P<0.001$). Based on RT-PCR results, goats in the unvaccinated control group shed more virus compared to all groups except for 1/12th ($P<0.05$), while goats in the full dose group shed less virus than goats in the 1/12th and the unvaccinated control group ($P<0.05$). The results suggest that the 1/3rd (0.67 ml) dose of the vaccine is sufficient to reduce viral shedding after heterologous challenge with a FMDV SAT1 pool.

Keywords: FMD, Vaccination, Dosage, Clinical protection, Goats

1. Introduction

Foot-and-mouth disease (FMD) is an acute, highly infectious and economically important transboundary animal disease that affects cattle, buffalo, pigs, sheep and goats [1]. The disease is caused by infection with FMD virus (FMDV), a small positive sense RNA virus in the genus *Aphthovirus*, family *Picornavirdae* [2]. Seven clinically indistinguishable serotypes of FMDV have been identified, namely, O, A, C, Asia-1 and Southern African Territories (SAT) 1, SAT2 and SAT3. Among these, serotypes O, A, C, SAT1, SAT2 and SAT3 have occurred in Africa, with serotype C last reported in Kenya in 2004 [3]. In southern Africa, FMDV serotypes SAT1, SAT2 and SAT3 are endemic in the African buffalo (*Syncerus caffer*), with sporadic outbreaks of SAT1 and SAT2 occurring in livestock [4–6]. The disease is characterized by fever, lameness and the appearance of vesicular and ulcerative lesions in the mouth, tongue, nose, feet and teats of lactating animals [7–9]. In goats, the clinical signs of FMD are typically considered mild or inapparent [10]. However, experimental infection can cause fever, nasal discharges, and development of ulcerative oral and interdigital cleft lesions [11].

Vaccination is an efficient and cost-effective method of infectious disease control in both human and animal populations [12]. However, a successful vaccination programme requires that the vaccine be of high quality and efficacious [13]. The overall goal of vaccination in the control of FMD can be broadly classified into four categories: reduction of clinical disease, elimination of circulating virus, maintenance of freedom from disease and regaining freedom from disease [13].

FMD vaccines are biological formulations containing one or more chemically inactivated cell-culture derived seed virus strain preparations blended with a suitable adjuvant and excipients [14]. Conventional FMD vaccines are formulated as either aqueous or oil-based preparations [15]. An

aqueous vaccine, which is mostly used in ruminants, is prepared by adsorbing the virus on to aluminum hydroxide gel and saponin. Oil-adjuvant vaccines are usually formulated using mineral oils [16]. While oil-adjuvants are in different forms, the FMD vaccines produced are mainly of the water-in-oil-in-water (W/O/W) form, which are generally formulated using Montanide ISA206 (Seppic, Paris, France) [17]. FMD vaccines can be classified as either "standard" or "high" potency. Standard vaccines are formulated to contain sufficient antigen to ensure the minimum potency, typically at least 3 PD_{50} (50% protective dose). High potency vaccines (>6 PD_{50}) are formulated with an increased amount of antigen to provide more rapid onset of immunity and a wider spectrum of immunity against closely-related field strains [14].

FMD vaccines are typically developed for use in cattle [18]. Vaccines are usually evaluated either by performing live animal challenge studies or by studying serological conversion, which correlates with protection in susceptible species [14]. Vaccine efficacy is the ability of a vaccine preparation to protect against disease, virus replication, virus shedding or virus transmission under controlled conditions [13]. SAT FMD vaccines have been evaluated for use in cattle and small stock [16,19–21]. Sheep and goats vaccinated with a trivalent (SAT1, SAT2 and SAT3) oil adjuvant vaccine maintained humoral antibody levels >1.6 $\log_{10}$ titres for up to 240 days for all three SAT antigens [20]. In a 1982 Nigerian study, cattle vaccinated with a trivalent (SAT1, SAT2 and A) vaccine were protected against a homologous intra-dermolingual challenge at 21 days post-vaccination [22]. Another study reported that an intra-serotype SAT2 chimeric FMD vaccine could induce strong neutralizing antibody titres that correlated with protection against homologous intra-dermolingual FMDV challenge in cattle [19]. SAT2 antigen from a thermo-stable and wild-type SAT2 oil-adjuvant vaccine induced full protection (absence of generalized FMD lesions) in all vaccinated cattle following a homologous intra-dermolingual challenge 5 months post-vaccination

[23]. An inactivated FMD vaccine was 80% effective clinically after a homologous SAT2 virus challenge in pigs, with no virus shedding occurring [24]. A high potency O_1 Manisa vaccine provided clinical protection following a homologous challenge in goats [25]. The same authors reported that one-half of the cattle dose of an oil-adjuvant vaccine is sufficient to induce protective immune responses in goats [26,27]. Other successful vaccination studies using FMDV serotypes O, A, C and Asia-1 have also been reported in goats [28–30]. The objective of the present study was to determine the efficacy of an inactivated double oil-emulsion FMD vaccine in indigenous South African goats challenged with a heterologous pool of SAT1 FMDV.

2. Materials and methods

2.1 Study design

The study was designed as a blinded randomized control study. Forty FMD sero-negative, indigenous South African goats (6-12 months of age) of mixed sexes were obtained from the FMD free zone of South Africa. Goats were stratified by sex and farm source and randomly allocated to one of five treatment groups using a computer-generated random number list. The calculated sample size for the study was 50 goats based on 80% power and 5% significance for the assumption of a response difference of 0.4 $\log_{10}$ titre values between vaccinated groups and a standard deviation (SD) of 0.3 for the control group [31]. However, considering the need for humane experimentation employing the concepts of the 3 Rs (replacement, reduction and refinement), the full dose and the control groups were reduced to 5 goats each and the final sample size was therefore 40 goats in total. The sample size was calculated based on the following formula [32].

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

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

μ_1 and μ_2 are the means of the two treatment groups

σ is the common standard deviation

$c = 7.9$ for 80% power

From the above, $\delta = 0.4/0.3 = 1.3$
And for 80% power, we had:

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

Five goats each were randomly allocated to the full cattle dose (2 ml) and the unvaccinated control (2 ml placebo) groups. Ten goats each were randomly allocated to the three reduced-dose treatment groups (1/3rd, 1/6th and 1/12th). The vaccine dose regimen was selected based on the recommended dose of 1/3rd cattle dose of alhydrogel-saponin and ½ cattle dose oil-emulsion FMD vaccine preparations for goats [15,33]. Animals were identified by unique identification ear-tags and allowed to acclimatize for 10 days in the BSL-3 animal facility at the Onderstepoort Veterinary Research, Transboundary Animal Disease, (OVR-TAD), Pretoria, South Africa, prior to the study. During the acclimatisation period, all animals were treated with 1% Noromectin® (Norbrook Laboratories, South Africa) at a dose of 5 mg/25 kg subcutaneously and Hi Tet 200 LA Gold® (Bayer Animal Health, South Africa) at a dose of 20 mg/kg deep intramuscularly. Some goats that were affected with infectious keratoconjuctivitis were treated with penicillin topically and Nuflor® (MSD Animal Health, South Africa) at 200 mg/kg intramuscularly for three consecutive days and repeated one week later. Animals were obtained from multiple sources with the possibility of passing through auctions prior to purchase and apparently arrived during the incubation period for respiratory and ocular infections. Animals were provided with *ad libitum* access to fresh drinking water, fed a complete pelleted ruminant ration once a day and housed in the BSL-3 animal facility at the OVR/TAD, Pretoria. All experimental protocols were reviewed and approved by the relevant authorities (AEC V022-17, University of Pretoria and AEC 6.17, Onderstepoort Veterinary Research). Permission for research in terms of the Animal Diseases Act, of the Republic of South Africa (Act No. 35 of 1984) was also obtained (DAFF 12/11/1/1, Department of Agriculture, Forestry and Fisheries).

2.2 Vaccine administration

The vaccine used was a complete blend of a high potency (>6 PD_{50} in 2 ml cattle dose) pentavalent vaccine containing SAT1 (SAR/9/81/1, BOT/1/106/1), SAT2 (KNP/1/10/2, SAR/3/04/2) and SAT3 (KNP/10/90/3) FMDV strains formulated with Montanide ISA 206 VG™ adjuvant (Seppic, France). The vaccine potency test was performed in a group of healthy FMD antibody-free cattle according to the OIE Terrestrial Manual [14] and the PD_{50} was calculated according to the Reed and Müench method [34]. The vaccine was produced by the OVR-TAD, South Africa for local field use. Goats were vaccinated on day 0 after the initial 10-day acclimation period and revaccinated on day 20 post initial vaccination. The vaccine was administered by intramuscular

injection of the left prescapular musculature using individual syringes and 18 G x 1 inch needles. Goats in the unvaccinated control group were administered 2 ml of antigen-free adjuvant as placebo *in lieu* of the vaccine preparation. One researcher not involved in clinical data collection (GTF) administered the vaccine and placebo and all researchers involved in data collection were blinded to treatment group assignment. Animals were housed in separate biosecure animal stables according to treatment group assignments.

2.3 Preparation and administration of challenge material

The challenge virus was a pool of three SAT1 (SAR/8/10/1, SAR/10/10/1 and SAR/21/10/1) FMDV isolated during a single outbreak in cattle within the FMD control zone of South Africa during 2010. Virus from field specimens were isolated in IB RS-2 (swine kidney) monolayer cell lines [35]. The challenge virus was prepared and adapted within two serial animal passages. For each viral passage, two goats and two Nguni cattle were inoculated with the pool of the FMDV SAT1 at a dose of $10^{4.5\text{-}5.5}$ $TCID_{50}$/ml. The challenge material for the second passage was prepared as a pool collected from both cattle and goats due to insufficient material from goats during the initial passage. The resultant lesion material from the goats only was used for challenging the goats in this study. The preparation of the host-adapted challenge material has been previously described [11,36].

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

to losses during the study (n = 3) and the inclusion of partially challenged (n = 1) and unchallenged (n = 2) in-contact sentinels.

2.4 Clinical scoring and specimen collection

All goats were monitored for 41 days post initial vaccination concerning their general health prior to challenge. Goats were physically examined daily post-challenge for signs of FMD and rectal temperatures recorded (temperatures ≥40°C defined as fever). The presence of vesicles and ulcerations on the tongue, lips, gums and feet in infected animals were recorded. Clinical signs of FMD were scored as previously described [26,37], with slight modifications: fever +1; each secondary lesion away from the site of inoculation +1. The total clinical score was determined by simple addition and each goat could theoretically score a maximum of 8 points: fever, secondary lesion on the tongue, gum, lip and each of the four feet.

Blood was collected from the jugular vein into plain evacuated tubes (Vacutainer®, BD Becton, Dickinson and Company, USA) before vaccination and thereafter on a weekly basis until the end of the study. Following challenge at day 41 post initial vaccination, oral, nasal and rectal swab specimens were collected from all animals on days 0, 2, 4 and 6 post-challenge using Puritan UniTranz-RT™ transport system (Puritan Diagnostics, USA). Blood was allowed to clot at room temperature and collected sera were stored at -20°C until testing. Swab specimens for FMDV RNA detection were stored at -70°C until testing. All goats were humanely euthanized by intravenous overdose of sodium pentobarbitone (Euthapent®, Kyron Laboratories) 14 days post-challenge. The FMD clinical descriptions of goats in this study have been presented elsewhere [11].

2.5 Serological assays

A solid-phase competition ELISA (SPCE) for FMDV serotype SAT1 was performed on collected serum samples following standard procedures [38,39]. Tests were performed in duplicate and final

optical density (OD) values were expressed as the percentage inhibition (PI) relative to the mean OD of the strong positive control wells. i.e. 100 – (100 x (OD test serum mean/OD strong positive control mean)). Samples with <50% inhibition were scored as negative and those ≥50% were considered positive [38].

Serum samples from 7 and 14 days post-challenge (euthanasia) were tested for the presence of antibodies against FMDV 3ABC non-structural proteins (NSP) using the PrioCHECK® FMDV NS (Prionics, Lelystad, Netherlands). Samples with PI<50% were classified as negative (antibodies against NSP considered absent) while samples with PI≥50% were classified as positive [40].

2.6 Real-time RT-PCR

FMDV RNA was extracted from clinical specimens using the QIAamp® RNA Viral Mini kit (Qiagen, Hilden, Germany) or the QIAamp® RNeasy Mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Real-time reverse transcriptase PCR (RT-PCR) for the detection of FMDV RNA in oral, nasal and rectal swab specimens was carried out using the iTaq™ Universal Probes One-Step Kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Primers targeting the 3D polymerase region of the FMDV genome were used; 3D forward (5' – ACT GGG TTT TAC AAA CCT GTG A – 3') and 3D reverse (5' – GCG AGT CCT GCC ACG GA – 3'). The probe was 3D probe (6-FAM 5' – TCC TTT GCA CGC CGT GGG AC – 3' TAMRA), [41]. The CFX96™ Real-Time PCR Detection system (Bio-Rad) was used and specimens with a cycle threshold ≤35 were considered positive.

2.7 Sequences analysis of FMD SAT1 challenge viruses

SAT1 challenge viruses (SAR/8/10/1, SAR/10/10/1 and SAR/21/10/1) were characterized according to RT-PCR and sequencing procedures described previously [42,43]. Nucleotide

sequences were submitted to the NCBI GenBank under the accession numbers MT227872, MT227873 and MT227874.

Partial VP1 nucleotide sequences were compiled in multiple sequence alignments using BioEdit v7.2.5 software [44] and CLUSTAL W [45]. A Neighbor-joining phylogenetic tree employing the p-distance method was constructed and visualised in MEGA5 [46] for the SAT1 challenge viruses and vaccine reference viruses. For evolutionary analysis, bootstrap values of the phylogenetic nodes were calculated out of 1000 replicates. The evolutionary divergence between SAT1 viral sequences was determined by the comparison of the number of base substitutions per site and was assessed in MEGA5 using pairwise analysis.

2.8 Statistical analysis

The normality assumption for all quantitative outcome variables was assessed by calculating descriptive statistics, plotting histograms and performing the Anderson-Darling test for normality using MINITAB Statistical Software, Release 16 (Minitab Inc, USA). SPCE antibody levels were presented as mean ± standard deviation and comparisons performed using one-way ANOVA. Correlation between SPCE mean PI and percentage clinical protection at the group level was estimated by Spearman's rho. The Kruskal-Wallis test was used to compare clinical scores across vaccine treatment groups. Rectal temperatures post challenge were compared among treatment groups using one-way ANOVA with Bonferroni correction of P values for multiple post-hoc tests. Quantitative variables were also compared at each day post-challenge using one-way ANOVA with multiple post-hoc tests adjusted using Bonferroni correction. Linear mixed models were fit to estimate the effect of treatment group on the quantitative outcomes of rectal temperature and viral RNA estimated from real-time RT-PCR (Ct value). Independent models were fit for each outcome in addition to a combined model for the three PCR specimens combined (nasal, rectal and oral swabs). All models included a random effect term for animal with a first-order

autoregressive (AR1) correlation structure to account for the repeated measurements. Fixed effects included terms for treatment group and days post challenge (dpc). Bonferroni correction was used to adjust P values for multiple post-hoc comparisons. Statistical analyses were performed in commercially available software (IBM SPSS Statistics Version 24, International Business Machines Corp., Armonk, New York, USA) and significance was set at $P<0.05$.

3. Results

3.1 Sequence analysis of FMD SATI challenge viruses

The SAT1 challenge viruses (SAR/8/10/1, SAR/10/10/1 and SAR/21/10/1) clustered with the SAT1 vaccine strains (SAR/9/81/1 and BOT/1/06/1) (Supplemental Figure 1) with average nucleotide identities of 82.1% and 76.4%, respectively.

3.2 Descriptive and clinical results

Forty goats were allocated to the five treatment groups at the beginning of the study with five goats each for the full cattle dose and the unvaccinated placebo control, while the $1/3^{rd}$, $1/6^{th}$ and $1/12^{th}$ groups were assigned ten goats each. Thirty-nine goats were vaccinated at the beginning of the study as one goat from the $1/12^{th}$ group died -6 dpv due to a pre-existing health condition. Two other goats died (one goat died at 17 dpv, from the $1/12^{th}$, and another goat at 38 dpv from the $1/3^{rd}$) before experimental challenge. All deaths were determined to be due to injuries or pre-existing conditions. Three goats from the $1/6^{th}$ group either received ½ the challenge dose (n = 1) or were maintained as sentinels (n = 2) to evaluate transmission and were subsequently excluded from the evaluation of the vaccine.

Goats in the unvaccinated control group had higher median clinical scores relative to all other treatment groups (Table 1). Goats in all vaccinated (full dose, $1/3^{rd}$, $1/6^{th}$ and $1/12^{th}$) groups had lower body temperature following virus challenge compared to the unvaccinated controls ($P<0.001$).

Table 1. Clinical onset of disease and median clinical scores of goats following intra-dermolingual challenged with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool.

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

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

In the 1/12th vaccine dose group, five goats developed muco-purulent nasal discharges between days 3 and 8 post-challenge. Four of the eight goats developed secondary lesions within 6-8 days post-challenge (Table 2). However, one goat never developed a secondary lesion or fever throughout the 14 day observation period.

Seven goats were challenged within the 1/6th vaccine dose group. None of the challenged animals in this group developed fever within the first 48 hours of challenge but by 72 h two goats had temperatures ≥40°C. In this group, one goat developed hyper-salivation at 3 day post-challenge without an obvious lesion at the site of inoculation or elsewhere on the oral mucosa. However, the oral swab from this goat tested positive for viral RNA at 4 days post-challenge and had a swelling at the site of inoculation on day 9. One other goat developed a secondary lesion at 8 days post-challenge (Table 2).

In the 1/3rd vaccine dose group, one goat did not develop lesions at the site of inoculation until day 5 post-challenge. The same goat developed interdigital lesions on both the left front and the right hind limbs at 7 days post-challenge (Table 2). One goat never developed any lesion at the site of inoculation or signs of fever throughout the 14 day observation period.

In the full dose vaccine group, four goats developed lesions at the site of inoculation within 48 h post-challenge, while one goat developed lesions at the site of inoculation 72 h post-challenge (Table 2). However, none of the goats developed secondary lesions. The level of protection provided against development of clinical disease appeared to be dose-dependent (Supplemental Figure 2).

Table 2. Summary of the clinical outcome in goats after intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool.

Days post challenge		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Groups	**ID**															
Group 1	**L1**	-	♦	-	-	-	-	-	-	-	-	-	-	-	-	
(Full cattle dose)	**L4**	-	†	♦	-	-	-	-	-	-	-	-	-	-	-	
	L9	-	♦ †	-	-	-	†	-	-	†	-	-	-	†	-	
	L19	†	♦	-	-	-	-	-	-	-	-	-	-	-	-	
	137	†	♦ †	-	-	-	-	-	-	-	-	-	-	-	-	
Group 2	**L5**	-	-	-	-	♦	-	■	■	-	-	-	-	-	-	
(1/3 cattle dose)	**L15**	†	-	♦	†	-	-	-	-	-	-	-	-	-	-	
	L18	-	♦	-	-	-	-	-	-	-	-	-	-	-	-	
	L20	-	♦	-	-	-	-	-	-	-	-	-	-	-	-	
	L29	†	†	♦ †	-	-	-	-	-	-	-	-	-	-	-	
	146	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	156	-	†	♦	-	-	-	-	-	-	-	-	-	-	-	
	162	-		♦	-	-	-	-	-	-	-	-	-	-	-	
	160	-	†	♦	-	-	-	-	-	-	-	-	-	-	-	
	L3*															
Group 3	**L8**	-	-		-	-	-	-	-	-	♦	-	-	-	-	
(1/6 cattle dose)	**L11**	-	-	♦	-	-	-	-	-	-	-	-	-	-	-	
	L12	-	-	†	♦	-	-	-	-	-	-	-	-	-	-	
	L16	-	-	-	-	-	-	-	-	■	-	-	-	-	-	
	L23	-	♦	-	-	-	-	-	-	-	-	-	-	-	-	
	132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	138	-	-	†	-	-	-	-	-	-	-	-	†	-	-	
	L28[e]															
	135[e]															
	161[e]															
Group 4	**L2**	-	♦ †	-	-	-	-	-	-	-	-	-	-	-	-	
(1/12 cattle dose)	**L14**	-	♦	-	-	-		-	-	†	-	-	-	-	-	
	L21	-	♦	-	-	-	■	-	-	-	-	-	-	-	-	
	L22	-	♦ †	†	-	-	-	■	†	-	-	-	-	-	-	
	L27	-	♦	†	-	-	-	-	■	-	-	-	-	-	-	
	140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	157	-	-	♦ †	-	-	-	-	-	-	-	-	-	-	-	

	158	†	♦ †	†	†	†	-	-	■	-	-	-	-	-	-
	L25*														
	152*														
Group 5	**L7**	†	♦ †	†	†	†	■	-	†	-	-	-	-	-	-
(Placebo)	**L10**	†	♦ †	†	†	†	-	-	-	-	-	-	-	-	-
	L17	-	♦ †	†	†	-	-	-	†	†	-	-	-	-	-
	L26	†	♦ †	†	†	†	†	†	■†	†	†	-	-	-	-
	166	†	-	†	♦ †	-	-	■	†	†	†	†	†	-	†

♦ = Lesion at the site of inoculation, ■ = Lesion at any other side including feet, mouth and tongue; indicative of generalized disease, † = Temperature ≥40°C, Animals L3*, L25* and 152* died before the challenge period, Animals L28[e], 135[e] and 161[e] were excluded from the challenge study.

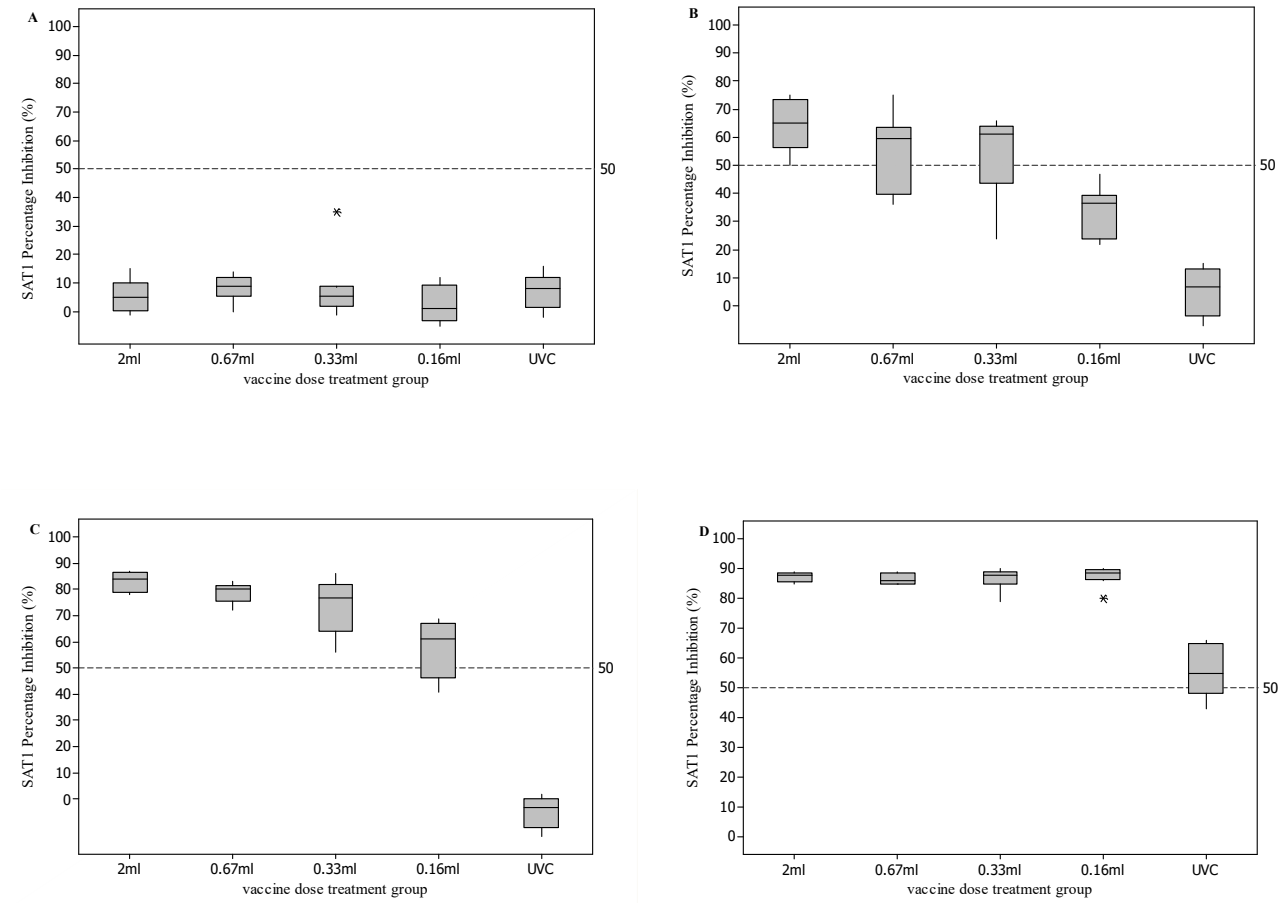

Figure 1. Descriptive presentation of SPCE FMDV SAT1 antibody levels across groups following vaccination showing median, first and third quartile values of antibody levels and the whiskers showing maximum and minimum levels: (A) FMDV SAT1 pre-vaccination sera at day 0, (B) FMDV SAT1 antibody levels at 20 dpv, (C) FMDV SAT1 antibody levels at 41 dpv (challenge day), and (D) FMDV SAT1 antibody levels 48 dpv (7 dpc).

3.3 Serological responses

All five goats in the full dose, 7/9 goats in the 1/3rd and 6/7 goats in 1/6th groups had seroconverted by 20 days post-vaccination with mean PI antibody levels reaching a peak of 65%, 55% and 53% for the three groups respectively (Figure 1; Supplemental Table 1). The serological differences among groups were not different statistically (P = 0.252). The peak average PI occurred at 27 days post-vaccination for all four vaccine treatment groups, with the full dose, 1/3rd and 1/6th groups reaching 86%, 84% and 83% mean percentage inhibition respectively. At seven days post-challenge, the mean antibody PI in all vaccinated groups had increased to >80% (P<0.001; Supplemental Table 2). There was a strong positive correlation between SPCE antibody levels and clinical protection (Spearman's ρ = 1; Figure 2). At study termination, all animals in the five treatment groups were seropositive to FMD SAT1 structural antibodies with mean antibody PI being highest for the full cattle dose group (Supplemental Table 2). All goats including the unvaccinated controls were positive for anti-3ABC non-structural protein antibodies at 7 days post-challenge (Table 3).

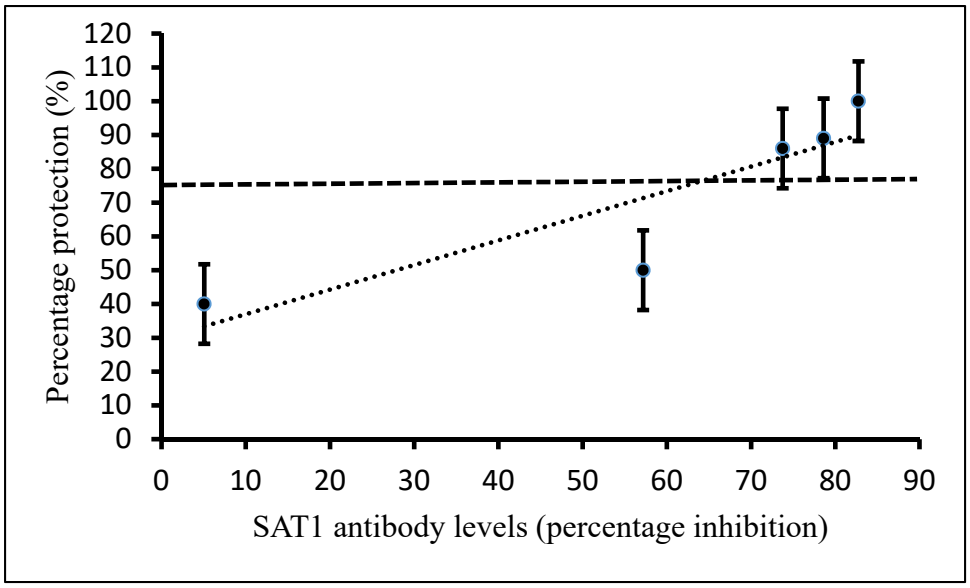

Figure 2. Correlation between SPCE antibody levels and clinical protection after intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool. Protection means animals that never developed secondary FMD lesions and a threshold of >75% is recommended for FMD

vaccines. Data presented are at the vaccine treatment group level with error bars representing the 95% CI of the mean.

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

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

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

3.4 Viral excretion

Three goats from the unvaccinated control group had viral RNA detected in nasal swab specimens at 2 days post-challenge, and by 6 days post-challenge all five goats had detectable viral genomic material (Table 4). Three goats from the 1/12th group had viral RNA in the nasal swab at 6 days post-challenge. However, none of the goats from the full dose, 1/3rd and 1/6th groups had evidence of viral RNA detected in the nasal swab specimens from 0-6 days post-challenge (Table 4). Viral RNA was detected from the oral swab of most goats from all five treatment groups by 48 h post-challenge and this extended until 6 days post-challenge for animals in the 1/12th and unvaccinated control group (Table 4). However, none of the goats in the 1/3rd group had viral RNA detectable from oral swab specimens beyond 2 days post-challenge, and only one animal each from the full dose and 1/6th group had viral RNA detectable from oral swabs at 6 days post-challenge (Table 4).

Two goats from the unvaccinated control group, and three goats from the 1/12th group had viral RNA detectable in rectal swab specimens at 2 days post-challenge (Table 4). By 4 days post-challenge, all five goats in the unvaccinated control group had detectable viral RNA in rectal swab specimens. Seven of the eight goats in the 1/12th group had viral RNA detected in rectal swab

Table 4. Summary of the FMD viral RNA in clinical specimens from goats after intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool.

Days post challenge		0	2	4	6	
Groups	**ID**					
Group 1	**L1**	-		O^+	O^+	O^+
(Full cattle dose)	**L4**	-		-	O^+	-
	L9	-		O^+	O^+	-
	L19	-		O^+	O^+	-
	137	-		O^+	-	-
Group 2	**L5**	-		-	-	-
(1/3 cattle dose)	**L15**	-		-	-	-
	L18	-		-	-	-
	L20	-		O^+	-	-
	L29	-		O^+	-	-
	146	-		O^+	-	-
	156	-		-	-	-
	162	-		-	-	-
	160	-		O^+	-	-
	L3*					
Group 3	**L8**	-		-	O^+	-
(1/6 cattle dose)	**L11**	-		O^+	R^+	-
	L12	-		-	O^+	-
	L16	-		O^+	O^+	-
	L23	-		O^+	O^+	O^+
	132	-		-	O^+	-
	138	-		-	O^+	-
	L28[e]					
	135[e]					
	161[e]					
Group 4	**L2**	-		O^+	$O^+ R^+$	O^+
(1/12 cattle dose)	**L14**	-		O^+	$O^+ R^+$	-
	L21	-		O^+	O^+	O^+
	L22	-		$O^+ R^+$	$O^+ R^+$	$N^+ O^+$
	L27	-		$O^+ R^+$	$O^+ R^+$	$N^+ O^+$
	140	-		-	$O^+ R^+$	$N^+ O^+$
	157	-		-	$O^+ R^+$	O^+
	158	-		$O^+ R^+$	$O^+ R^+$	O^+
	L25*					
	152*					
Group 5 (Placebo)	**L7**	-		$N^+ O^+$	$O^+ R^+$	$N^+ O^+$
	L10	-	R^+		$N^+ O^+$	$N^+ O^+$
	L17	-		$N^+ O^+$ R^+		$N^+ O^+$
	L26	-		O^+	$O^+ R^+$	$N^+ O^+$
	166	-		O^+	$N^+ O^+$	$N^+ O^+$
				$N^+ R^+$ R^+		
					$O^+ R^+$	

N^+ = Viral RNA detected in nasal swab specimen, O^+ = Viral RNA detected in oral swab specimen, R^+ = Viral RNA detected in rectal swab specimen, Animals L3*, L25* and 152* died before the challenge period, Animals L28[e], 135[e] and 161[e] were excluded from the challenge study.

Table 5. Multivariable model estimates of fixed effects of vaccine treatment group for goats following intra-dermolingual challenged with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool on the quantity of virus recovered from nasal, rectal and oral swabs.

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

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

specimens with one goat from the 1/6th group at 4 days post-challenge. No viral RNA was detected from rectal swab specimens beyond 4 days post-challenge. There were significant effects of vaccine treatment group and specimen type on the magnitude of FMDV shedding (Table 5). Viral shedding of goats in the 1/12th vaccine dose group did not differ significantly from the shedding of FMDV from the unvaccinated control goats (P = 0.948). However, there was a significant difference between the three larger vaccine dosages (full dose, 1/3rd and 1/6th) and the other two groups (P<0.001). Goats in the unvaccinated control group shed more virus compared to all vaccine groups except the 1/12th dose.

4. Discussion

Vaccination is an important tool for the prophylactic control of FMD in endemic settings, where the goal is to reduce clinical disease and economic losses in livestock rather than eradication [13]. The efficacy of a vaccine can be determined by using animal challenge studies based on reduction of clinical disease manifestation and viral shedding as detected by either virus isolation in cell culture or viral RNA on RT-PCR. It is recommended that the efficacy of FMD vaccines should be >75% compared to the unvaccinated control group based on protection against podal generalization [14]. The aim of this study was to determine the efficacy of an experimental double-oil emulsion FMD vaccine against disease and viral shedding in vaccinated and challenged goats. To our knowledge, this is the first study to evaluate the efficacy of a SAT serotype FMD vaccine in goats. Vaccination induced SAT1 antigen-specific immune responses as early as 14 days post-vaccination, with antibody levels for the three vaccine groups (full dose, 1/3rd and 1/6th dose) reaching the antibody threshold level by 20 days post-vaccination. Vaccination also provided dose-dependent clinical protection among vaccinated groups with fewer secondary FMD lesions and reduced viral shedding compared to the unvaccinated group. This is similar to the results from a heterologous challenge study after vaccination with a high potency vaccine and challenge with

serotype Asia-1 FMDV. In the previous study, vaccination reduced excretion of virus in nasal and oral secretions of sheep following intra-nasopharyngeal challenge [9].

In the current study, there was a high proportion of sero-conversion in three of the vaccinated groups (full dose, $1/3^{rd}$ and $1/6^{th}$) by 20 days post-vaccination just prior to revaccination. Peak mean antibody levels of 86%, 84%, 83% and 77% occurred at 27 days post-vaccination in all four vaccine treatment groups respectively. This finding is consistent with a previous study using an oil-adjuvant vaccine in goats [30]. A similar study in sheep also described higher FMDV Asia-1 specific antibody levels by SPCE as early as 21 days post vaccination with a high potency vaccine [9]. In the present study, all vaccinated groups had mean SPCE antibody levels above the positive threshold at the time of challenge, while all unvaccinated goats remained sero-negative. High potency FMD vaccines are known to induce rapid immune responses in sheep [47]. In our study, there was a strong positive correlation between serological responses and clinical protection following challenge. Clinical protection against FMD has been previously reported to be associated in part with the induction of a serum antibody responses in sheep [48].

Non-structural protein antibody responses were detected in both vaccinated and unvaccinated animals as early as 7 days post-challenge. This is not a surprise for the vaccinated animals, where a rapid anamnestic response is expected and the employed vaccine might not be highly purified and completely NSP-free. However, the appearance of NSP antibodies in the unvaccinated controls 7 days post-challenge is sooner than the 10-35 day range previously published for experimental infections in goats [26]. Although, in a study to evaluate the performance of a SAT serotype-specific 3ABC assay using specimens from cattle, three NSP assays (PrioCHECK®-NSP, IZSLER-NSP and SAT-NSP) detected NSP antibodies at 5-7 days post-infection with SAT1 and SAT3 viruses with the exception of SAT1/NIG/5/81 infected animals which later tested positive

at 14 days post-infection [49]. Antibodies to NSP have also been detected as early as 7-10 days in pigs following a FMDV O Taiwan challenge [50].

Goats that received the 1/12th dose were not protected against disease relative to higher vaccine dosages (1/3rd and 1/6th). However, goats receiving the 1/3rd cattle dose had good protection relative to the 1/6th and 1/12th vaccine groups. Intriguingly, one goat infected with the ½ virus challenge dose in the 1/6th group remained FMD viral RNA negative for all specimens and NSP free without any signs of FMD throughout the study period. However, natural transmission occurred in the two sentinels maintained in this group [11]. These results suggest that vaccination in combination with the reduced challenge dose induced protective immunity in this goat.

High potency O_1 Manisa FMD vaccines reduce virus excretion following homologous challenge in goats compared to unvaccinated controls [25]. In this study, there was low viral shedding from clinical specimens collected from three vaccine groups (full dose, 1/3rd and 1/6th), which might be a result of the dampening effect of vaccination. There was no evidence of viral shedding from nasal epithelium of goats in the higher vaccination treatment groups (full dose, 1/3rd and 1/6th) and only 3 goats in the 1/12th group had viral RNA detected in nasal swab specimens at 6 days post-challenge. Furthermore, there was no evidence of viral RNA excreted in rectal swab specimens beyond 4 days post-challenge. Since the goats were infected by the intra-dermolingual route, detection of viral RNA in oral swab specimens should not be considered a strong indication of systemic viral shedding, even though this drastically reduced to only two goats in the full dose and 1/6th dose vaccine groups by 6 days post-challenge. The hyper-salivation observed at 3 days post-challenge in a goat within the 1/6th group might be a result of viral replication in the mucosal tissues since the same goat tested positive for viral RNA in an oral swab specimen collected at 4 days post-challenge. Swelling also appeared at the site of inoculation at 9 days post-challenge.

Vaccination of goats with low antigen payloads of an oil-adjuvant O_1 Manisa vaccine followed by homologous challenge reduced virus replication in the oropharynx, shedding of virus in nasal secretions and reduced the amount of virus released into the environment [26]. One-half cattle dose of a high potency vaccine induced protective immune responses in goats after a homologous FMDV O_1 Manisa direct in-contact challenge [27]. Our results suggest that vaccine doses less than the one-half cattle dose might be sufficient to reduce FMDV transmission in goats. This would be advantageous due to the reduction in cost when vaccinating large populations of animals in endemic settings.

In most FMD vaccine efficacy studies conducted in cattle and sheep, protection from clinical disease did not always coincide with prevention of localized, subclinical infection. FMDV has been previously detected within the oropharynx of 50% of vaccinated goats within the first 10 days post challenge [26]. In this study, we observed less viral RNA from oropharyngeal specimens in the larger dose vaccinated groups (full dose, $1/3^{rd}$ and $1/6^{th}$), which might suggest the ability of the vaccine to either prevent or reduce virus replication at the site of primary infection (oropharynx). This could theoretically reduce the amount of infectious material released into the environment from sub-clinically infected goats.

It is usual practice to employ a homologous challenge virus in FMD vaccine efficacy studies [51]; however, we employed a pool of heterologous SAT1 2010 FMD viruses recovered from different time points during a single outbreak in cattle. Therefore, protection observed with this vaccine might have been higher if a homologous virus challenge was employed to the SAT1 strains in the vaccine. However, the SAT1 viruses used as a pool of the field challenge clustered closely to the SAT1 vaccine strains.

The results of this study should be evaluated in light of several limitations. The small number of animals allocated to each treatment group reduces the precision of our estimates. However, considering the principle of humane experimental technique employing the replacement, reduction and refinement concepts, working in a containment facility with animals requires minimum numbers for welfare reasons. In order to reduce the possibility of confounding, a stratified random allocation process was employed. Though, the limited number of goats available for the study in addition to the losses that occurred suggests that confounding still could have impacted reported results. Blinding was employed to limit information bias, especially in relationship to the clinical scoring. The different group sizes in conjunction with housing groups within independent animal rooms allowed blinded researchers to determine which two rooms housed the control groups versus the three rooms that contained the lower vaccine dosages. However, the development of primary FMD lesions at the site of experimental infection occurred within all treatment groups and it was therefore not possible for blinded researchers to determine individual group assignments. To improve the external generalizability of the results, animals of mixed sexes, body sizes and source were included in the study. Efficacy was determined based on the reduction of clinical disease and viral shedding only and it is a limitation that viraemia was not compared between groups. This study is also limited by the fact that we did not titrate the virus in goats to determine the optimal challenge dose prior to the current study. The paucity of clinical signs in some groups, especially the $1/6^{th}$ cattle dose, suggests that the challenge system requires further refinement. Other limitations include the reliance on RT-PCR without virus isolation confirmation and not incorporating virus neutralization tests as an additional outcome to compare among treatment groups. Furthermore, it would have been advantageous to include unchallenged sentinel goats in all treatment groups to determine the potential of vaccination to prevent natural transmission.

A fractional dose of one-third (1/3rd) the full cattle dose of a high potency double oil-emulsion FMD vaccine containing SAT1 virus strains can confer protection in goats against a heterologous challenge with a SAT1 FMDV pool. However, there is a need to further study the effect of the vaccine preparation on virus replication and duration of immunity after vaccination. Additionally, the evaluated vaccination schedule might not be feasible for use in endemic situations and further research is required to identify a cost-effective approach to vaccinating goats in southern Africa. It also is important to study other breeds of goats since exotic breeds are considered to be more susceptible to FMDV compared to animals that are indigenous to areas where FMD is endemic [52]. Presented information advances our knowledge of vaccine performance in goats, which should improve the progressive control of FMD in southern Africa.

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Competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/ or publication of this article.

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Supplementary Material

Supplemental Table 1. Earliest time point of seroconversion by SPCE Mean ± Standard Deviation PI (%) in goats vaccinated with a pentavalent FMD vaccine at day 0 and revaccinated at 20 dpv.

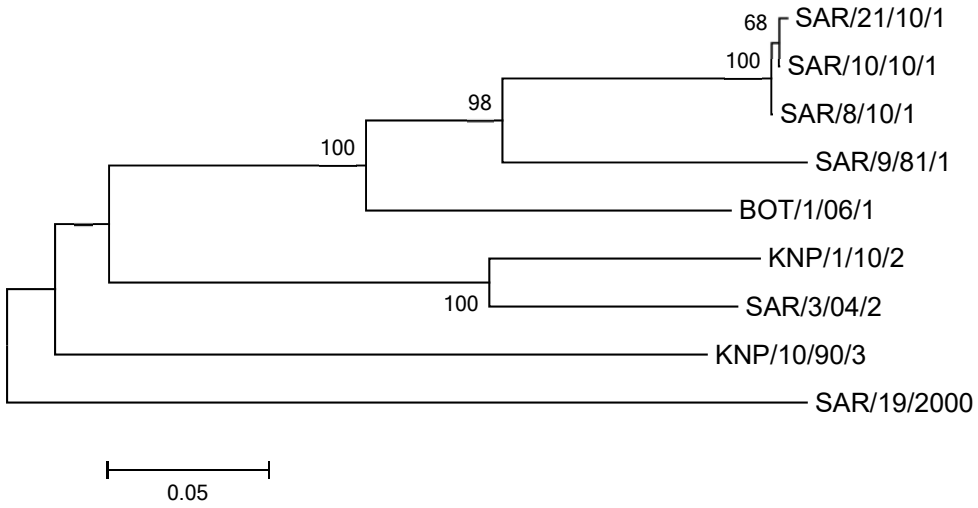
SAT1 Vaccine Group	dpv	Mean ± SD	P value*
Group 1 (2 ml)	7	22.24 ± 12.06	0.010
	14	63.18 ± 10.08	
	20	65.11 ± 9.82	
	27	85.98 ± 2.70	
	34	83.75 ± 2.15	
Group 2 (0.67 ml)	7	24.16 ± 10.27	<0.001
	14	49.65 ± 14.34	
	20	55.45 ± 12.79	
	27	83.94 ± 2.05	
	34	81.31 ± 2.66	
Group 3 (0.33 ml)	7	15.41 ± 10.47	<0.001
	14	44.16 ± 11.38	
	20	53.11 ± 14.41	
	27	83.13 ± 4.84	
	34	80.96 ± 3.18	
Group 4 (0.16 ml)	7	4.89 ± 9.72	0.066
	14	30.53 ± 13.62	
	20	33.60 ± 8.91	
	27	76.66 ± 4.99	
	34	72.03 ± 5.76	
Group 5 (unvaccinated placebo)	7	8.03 ± 7.77	0.732
	14	11.36 ± 8.82	
	20	5.36 ± 8.86	
	27	5.98 ± 6.94	
	34	5.07 ± 4.37	

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

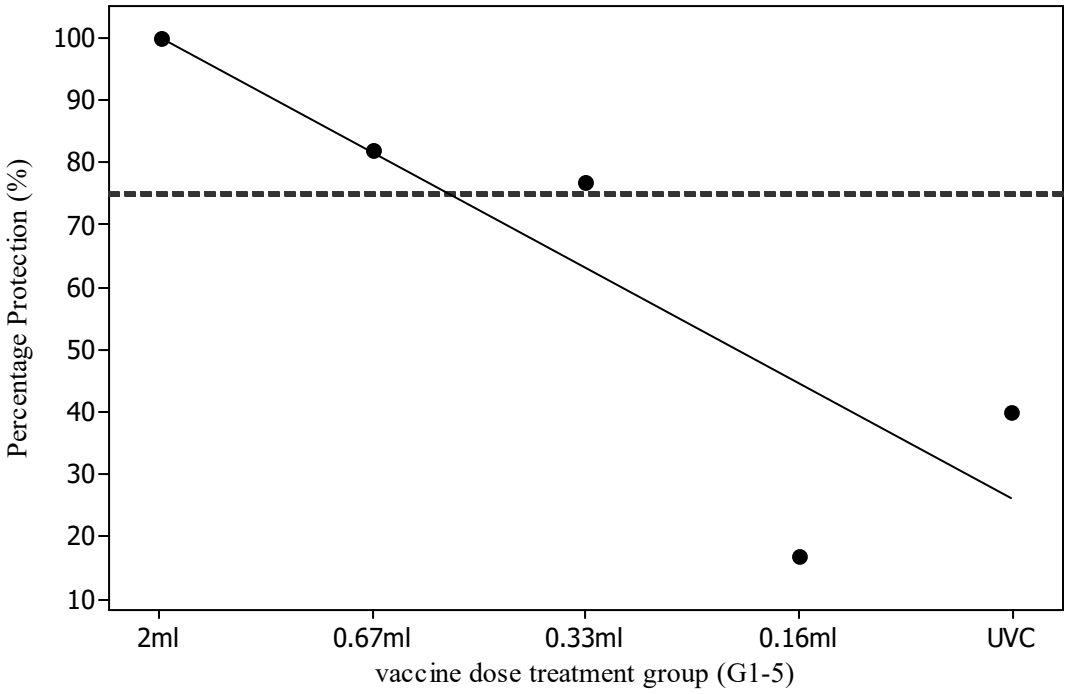
Supplemental Table 2. Peak antibody levels by SPCE Mean ± Standard Deviation PI (%) attained on the day of challenge (41dpv) and antibody responses following intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool from 48 to 55 days post-vaccination.

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

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



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



Supplemental Figure 2. Correlation between vaccine dose treatment group and percentage protection after intra-dermolingual challenge of goats with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool. Protection means animals that never developed secondary generalized FMD lesions and a threshold of >75% is recommended for FMD vaccine efficacy test.