Clinical presentation of FMD virus SAT1 infections in experimentally challenged indigenous South African goats

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Highlights

•FMDV infection was evaluated in indigenous South African goats after experimental challenge with SAT1 virus pool.
•FMDV SAT1 causes mild clinical disease in indigenous goats characterized by fever, ulcerative oral and hoof lesions.
•Experimentally challenged goats developed nasal discharges, which has not been previously reported.
•Natural transmission of FMD occurred between challenged goats and vaccinated unchallenged in-contacts.
•There is a need to further investigate the role of goats in the epidemiology and transmission of FMD in southern Africa.

Abstract

Foot-and-mouth disease (FMD) is a transboundary animal disease that has a major impact on livestock production and trade. Foot-and-mouth disease virus (FMDV) is a single-stranded RNA virus that infects cloven-hoofed livestock and wildlife. The susceptibility of South African indigenous goats to FMDV Southern African Territories 1 (SAT1) was investigated after experimental challenge with a mixed SAT1 virus pool. In this study, we present the clinical manifestation of FMDV in five naive goats challenged via the intra-dermolingual route with $10^{4.57}$ 50% tissue culture infective dose ($TCID_{50}$) FMDV virus pool containing SAT1 SAR/8/10, SAR/10/10 and SAR/21/10. The clinical responses of two vaccinated unchallenged goats maintained as in-contacts are also presented. Clinical scoring of FMDV infection and daily rectal temperatures were recorded and temperatures ≥40°C were defined as fever. All five challenged goats developed fever within 48 hours post challenge with a median fever duration of 5 days. The two unchallenged goats developed fever at 5 and 9 days post-contact with FMD lesions appearing at 4 and 8 days post-contact. Additional clinical signs observed included nasal discharge, ulcerative oral mucosal lesions of the lip and ulcerative interdigital cleft lesions. The pooled FMDV SAT1 infection caused mild clinical signs and natural transmission to reduced-dose vaccinated in-contact indigenous South African goats occurred.

Keywords

Clinical
Experimental
Foot-and-mouth disease
Infection
Goats

1. Introduction

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

The clinical signs of FMD in goats are considered to be mild but clinical descriptions have not been previously reported. Antibodies against FMDV non-structural proteins suggestive of viral exposure in unvaccinated animals has been reported previously (Balinda et al., 2009; Bhebhe et al, 2016; Habiela et al., 2010; Hyera et al., 2006; Lazarus et al., 2012). In the Southern African Development Community (SADC), the African buffalo (*Syncerus caffer*) is the wildlife reservoir host maintaining SAT1, SAT2 and SAT3 (Paton et al., 2018; Thomson et al., 2003; Vosloo and Thomson, 2017). FMD outbreaks within the SADC have increased in frequency and in many situations, these outbreaks have persisted for a longer time (Jori et al., 2016; Penrith and Thomson, 2012). Traditional FMD control measures have become inadequate in some parts of the SADC during the last 10-15 years (Lazarus et al., 2018; Thomson et al., 2013; Vosloo and Thomson,

2017). Several countries in the SADC have reported outbreaks during the past decades, with South Africa officially reporting FMD outbreaks within the FMD free zone of the country in February 2011 and January 2019 (DAFF, 2019; OIE-WAHID, 2018, 2017; Vosloo and Thomson, 2017).

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

2. Materials and methods

2.1 Ethics statement

This study was approved by the University of Pretoria, Animal Ethics Committee (V022-17) and the Onderstepoort Veterinary Research Animal Ethics Committee (AEC 6.17). Approval in terms of the Animal Disease Act (Act No. 35 of 1984) was obtained from the National Department of Agriculture, Forestry and Fisheries: Directorate of Animal Health, Republic of South Africa.

2.2 Preparation of FMDV SAT1 virus pool challenge material

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

from the first pooled virus challenge was used to challenge a second set of two goats and two cattle. Clinical material (epithelial lesions from the mouth and feet) from the second set of goats was again collected and pooled as previously described and prepared as the challenge material for the current study.

2.3 Experimental animals

A group of 40 indigenous South African goats (6-12 months of age) were sourced from livestock farms within the FMD free zone of South Africa prior to the 2019 FMD SAT2 outbreak (DAFF, 2019) for the evaluation of an inactivated oil-emulsion FMD vaccine (data not presented). The emphasis of this paper is only the clinical descriptions of the five unvaccinated control goats and two vaccinated unchallenged in-contact goats maintained during the study. The two in-contact goats were vaccinated with a reduced dose ($1/6^{th}$ cattle dose) of the oil-emulsion FMD vaccine containing serotypes SAT1, SAT2 and SAT3.

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

2.4 Clinical scoring

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

2.5. Sample collection and processing

Clotted blood for serology was collected on day 0 before animals were vaccinated and at termination into plain evacuated tubes (Vacutainer®, BD Becton, Dickinson and Company, USA). Samples were allowed to clot at room temperature and sera harvested and stored at -20°C until testing. Heparinised blood was collected at 0, 2, 4 and 6 days post challenge into sodium heparin (Vacutainer®, BD Becton, Dickinson and Company, USA) for virus detection and stored at -70°C until testing. Epithelial tissue from fresh lesions were collected into a specimen bottle with Roswell Park Memorial Institute (RPMI) 1640 media (Sigma-Aldrich) and stored at -70°C until testing. Oropharyngeal specimens were collected from all goats at 6 days post challenge using a small ruminant probang cup and samples stored in RPMI-1640 media (Sigma-Aldrich) at -70°C until testing.

2.6 Laboratory analysis of specimens

2.6.1 Solid-phase competition ELISA (SPCE)

A SPCE for FMDV serotype SAT1 was performed following standard procedures (Paiba et al., 2004; Mackay et al., 2001). Tests were performed in duplicate and the final optical density (OD) values were expressed as the percentage inhibition (PI) relative to the mean OD of four strong

positive control wells. i.e. 100 – (100 x (OD test serum mean/OD strong positive control mean)). Samples that showed <50% inhibition of the OD strong positive control were classified as negative and those ≥50% were considered a positive serological response (Paiba et al., 2004). SPCE is a serotype-specific serological assay with a sensitivity of 100% for FMDV serotypes O, A and C (Mackay et al., 2001) and a specificity of 99% for SAT serotypes (Li et al., 2012).

2.6.2 Quantitative real-time RT-PCR

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

3 Results

3.1 Clinical outcomes

All five goats challenged with the FMDV SAT1 pool developed elevated temperatures within 48 hours with a median fever duration of 5 days (Figure 1). One goat (L26) had fever that lasted for 10 consecutive days. Four goats had tongue lesions at the site of inoculation 72 hours post challenge (Figure 2, top left). Animal L10 developed a tongue lesion two days post challenge and presented with bilateral nasal discharge on day 3, which lasted for three days (Figure 2, top right). Animal 166 developed a tongue lesion on day 4 at the site of inoculation and a secondary lesion

of the ventral oral lip on day 7 (Figure 2, bottom left). Animal L26 developed a tongue lesion on day 2, nasal discharge on day 3 and left front hoof and right front hoof interdigital cleft lesions on day 8. Animal L7 developed a tongue lesion on day 2 and a right hind limb hoof lesion on day 6 (Figure 2, bottom right). Animal L17 only developed a tongue lesion on day 2 post challenge. One of the vaccinated unchallenged in-contact goats (L28) developed fever on in-contact day 7, which lasted for three consecutive days. The other goat (161) developed fever on in-contact day 5 that only lasted for 1 day (Figure 3). Animal 161 developed an ulcerative lesion on the lip at in-contact day 4 and the other goat (L28) developed a similar lip lesion on day 8. The maximum clinical score for the challenged goats was three on days 8, 9 and 10 post-challenge (Table 1). Clinically apparent lameness was not identified and none of the goats lost weight or had a reduced appetite at any time during the study.

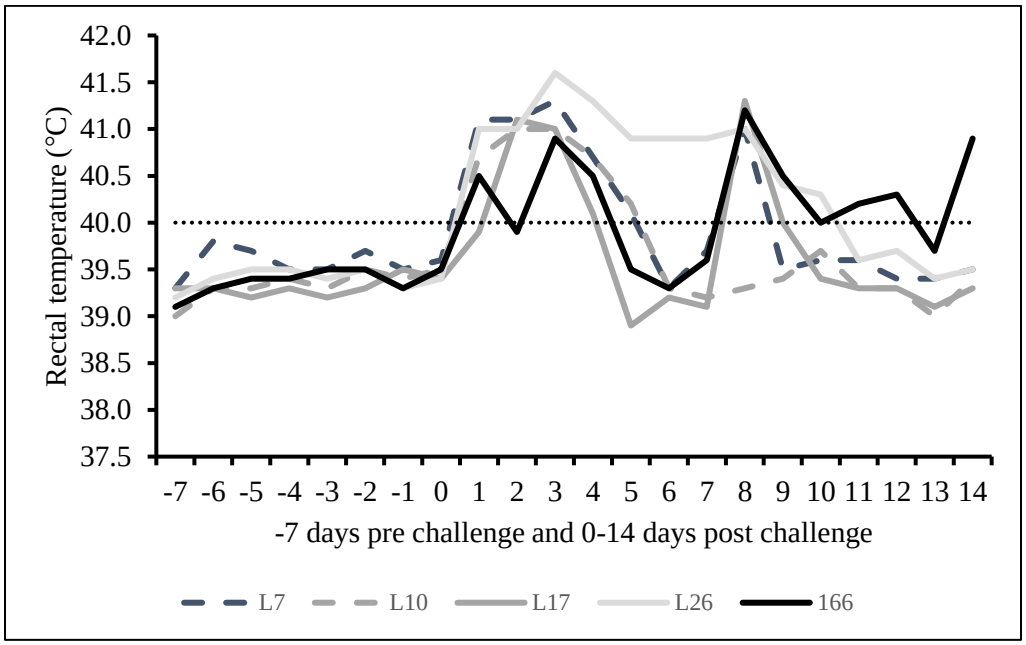

Figure 1. Rectal temperatures -7 days pre challenge to 14 post challenge of goats intra-dermolingually inoculated with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool. Probang samples (oropharyngeal specimen) were collected at day 6 post challenge. Fever was defined as a temperatures ≥ 40°C.

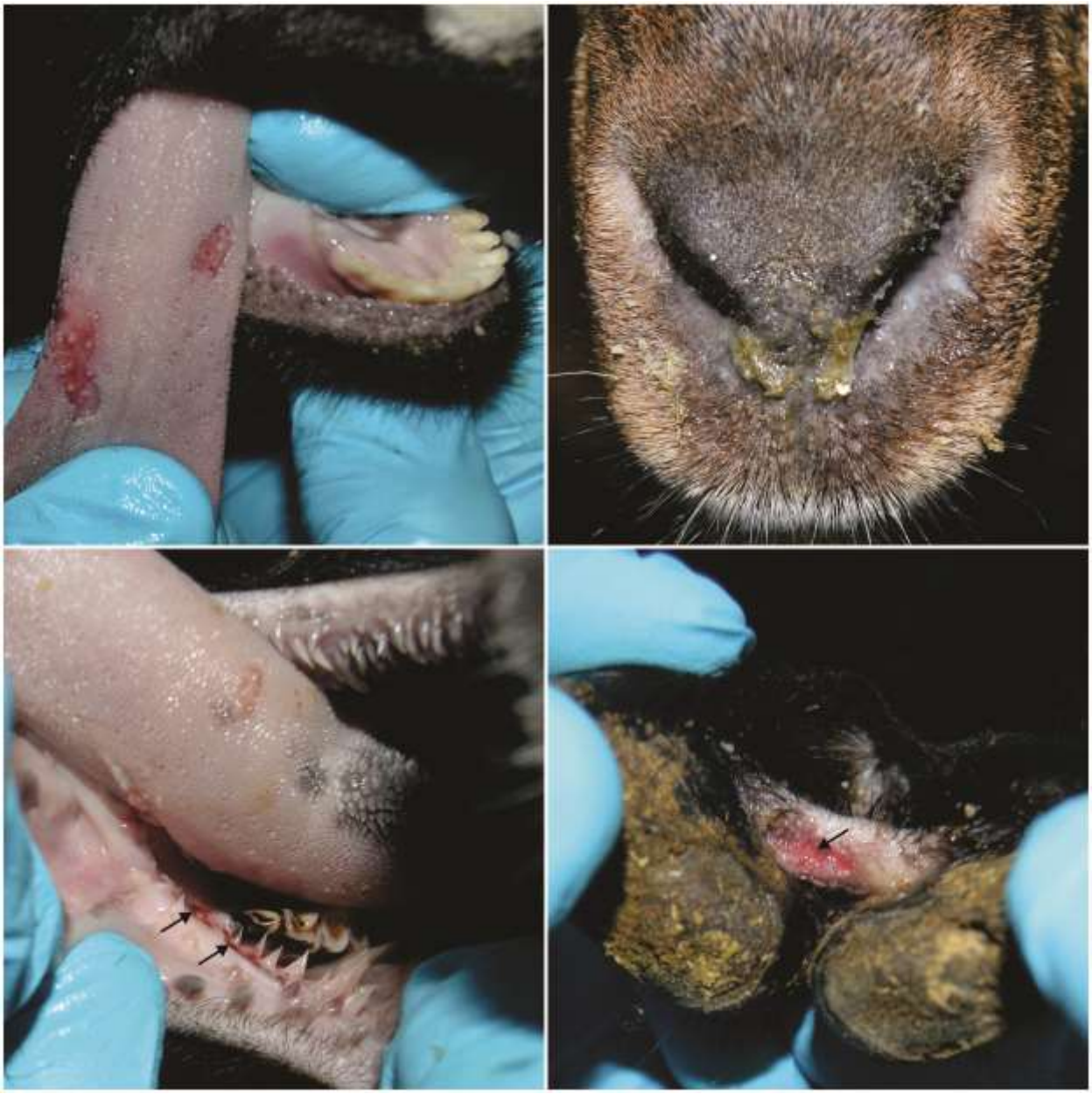

Figure 2. Top left: Ulcerative tongue lesion at the sites of inoculation 48 h post challenge of an indigenous South African goat with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool. Top right: Bilateral nasal discharge 3 days post challenge. Bottom left: Ulcerative lesion on the oral mucosa of the ventral lip. Bottom right: Interdigital cleft lesion 6 days post challenge.

Table 1. Clinical lesion scores of five goats following intra-dermolingual challenge with $10^{4.57}$ $TCID_{50}$ FMDV SAT1 pool and two unchallenged goats maintained in direct contact with experimentally infected goats.

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

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

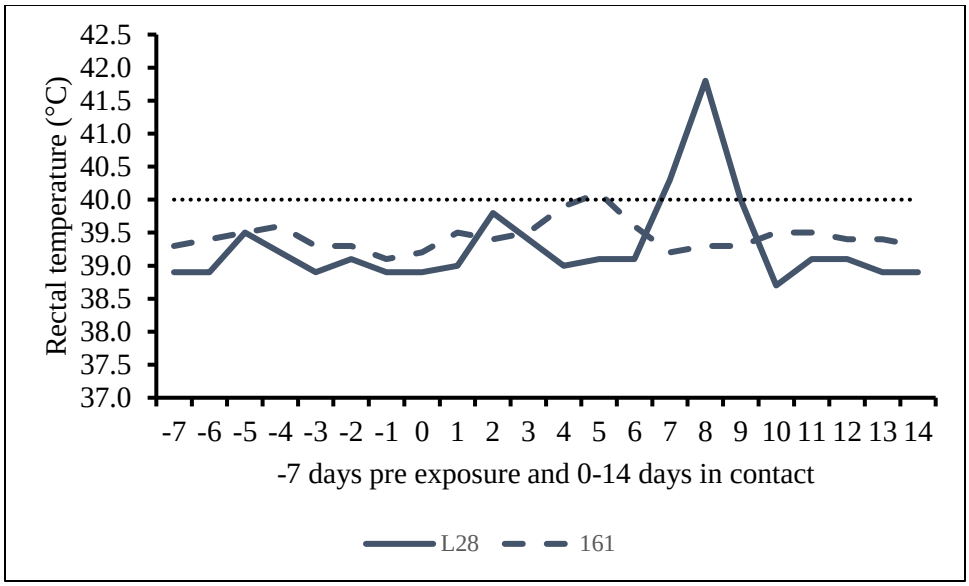

Figure 3. Rectal temperature -7 days pre-exposure and 0-14 days in contact for the two unchallenged goats maintained with the challenged goats. Fever was defined as a temperature ≥40°C.

3.2 Antibody responses

SPCE against SAT1 viruses were negative in all goats at the beginning of the study with a mean ± SD percentage inhibition (PI) of 5 ± 6 (Table 2). At termination (55 days) of the study, all goats were FMDV SAT1 seropositive with a mean ± SD PI of 83 ± 7.

Table 2. Solid phase competition ELISA (SPCE) percentage inhibition (PI) values for five goats experimentally infected with a pool of foot-and-mouth disease Southern African Territories 1 viruses and two in-contact exposed goats at the beginning and termination of the study.

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

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

3.3 Virus detection

All lesion materials (epithelial tissues) collected from challenged goats tested positive for FMDV RNA by RT-qPCR (Table 3). However, only one sample of epithelial material tested positive from the two in-contact goats. FMD viral RNA was detected in heparinized blood samples of three challenged goats at 2 days post challenge and two goats at 4 days post challenge. All animals were positive for viral RNA in oropharyngeal specimens.

Table 3. Foot-and-mouth disease (FMD) viral detection in clinical specimens as determined by RT-qPCR after challenge with FMDV SAT1 pool (in challenged goats) and unchallenged in-contact goats.

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

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

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

4 Discussion

The clinical signs observed in this study were consistent with what has been reported for sheep (Zaikin, 1959; Littlejohn, 1970; Kitching and Hughes, 2002). The most prominent signs were fever, ulcerative oral and hoof lesions. The second peak in rectal temperatures in all challenged animals followed oropharyngeal sampling on day 6 post challenge, which was likely associated with the stress of sedation and animal handling. The two vaccinated unchallenged goats maintained during the study only developed oral lip lesions following natural transmission via direct contact. This was similar to our field observations where ulcerative oral lesions were observed in goats during the recent South African SAT2 outbreak. This outbreak in cattle was confirmed by RT-qPCR but virus was not detected in the sampled goats. Only serological evidence of FMDV

exposure was identified in sampled small ruminants (data not presented). In both the epithelial tissues of the goat observed from the field and one of the vaccinated unchallenged goat that had lip lesions, no viral RNA was detected in the specimens even though the lesions were consistent with FMD. There seems to be no biological explanation as to why the two specimens tested negative by RT-qPCR, while the rest of the specimens tested positive using the same assay.

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

The clinical signs of FMD appeared in both the challenged and unchallenged goats between 4-8 days. This is similar to previous reports of a 2-8 day FMD incubation period in sheep and goats (Kitching and Hughes 2002; McVicar and Sutmoller 1972). However, this variation in timeline might depend on susceptibility of the host species, challenge virus dose as well as the route of infection. Infection with this pooled mixture of FMDV SAT1 only caused mild clinical lesions in our study goats. Clinical findings were classified as mild since the goats did not become anorexic or lame and observed lesions were less severe than what has been typically reported for cattle and sheep. We are uncertain if goat breed or the administered SAT1 FMDV pool of viruses influenced the clinical presentation in our study animals. Viraemia typically occurs 24-30 hours following

intranasal inoculation in sheep and lasts for 1-5 days (Hughes et al., 2002). As in cattle and pigs, fever and vesicles have been described to be the hallmark of clinical FMD in small ruminants and this has been reported to occur within 12-48 hours after the onset of viraemia (Arzt et al., 2011). This is similar to the results of the present study where viraemia was detected 2-4 days post challenge with clinical signs appearing after the viraemic phase. Also, aerosol shedding of the virus in sheep reaches a peak before the onset of clinical signs (Alexandersen et al., 2002; Burrows, 1968).

Some experimentally challenged goats developed nasal discharge, which has not been previously reported. However, following infection, FMDV replicates within the pharyngeal tissues and there is evidence that primary replication might occur in the nasal mucosa of sheep (Arzt et al., 2011). Oral lesions might occur more commonly in goats relative to sheep with some strains of FMDV (Olah, 1976); however, in field outbreaks affecting both sheep and goats, clinical signs are often reported to be more mild in the later (Arzt et al., 2011). One goat that presented with clinical signs before the development of fever also suggests that sub-clinically infected goats might shed virus silently without obvious signs of disease. Consequently, when inspecting goats for suspected FMD infections, attention should be focused on the oral mucosa of the lips and gums in addition to the tongue. For improved diagnostics, there is the need to further evaluate the performance of the RT-qPCR for the detection of FMDV clinical specimens in goats. There is also a need to investigate the role of goats in the epidemiology and maintenance of FMDV under field conditions in southern Africa.

This was a small experimental animal challenge study performed to evaluate vaccine efficacy (data not presented) and results are limited by the small number of animals. However, presented results improve our knowledge of the clinical presentation of SAT1 FMDV infections in goats after

experimental challenge and natural transmission. Another limitation of the study is the use of vaccinated unchallenged goats instead of naïve goats for the evaluation of natural transmission. We are also unable to present viraemia data for the unchallenged goats even though we had data on fever and clinical presentations. The research is ongoing and we are currently uncertain which of the viruses in the pool were responsible for disease. Future genetic evaluation of recovered viruses is a part of the research programme and these findings should answer this question. Continued research is necessary because an understanding of the epidemiological role of non-cattle livestock will improve the progressive control of FMD in southern Africa.

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Competing interests

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

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