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Article history

Submitted: 29-03-2023, Revised: 11-03-2024, Accepted: 07-09-2024, Published: 29-09-2024

Tanzania Veterinary Journal Vol. 39(1) 2024 ***https://dx.doi.org/10.4314/tvj.v39i1.3***

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SUMMARY

Foot and Mouth Disease Virus (FMDV) causes a highly devastating Foot and Mouth Disease (FMD) to all cloven hoofed livestock and wildlife. FMDV circulates worldwide as seven antigenically distinct serotypes (O, A, SAT1-3, C, and Asia1), and African buffalo act as reservoir of SAT1-3 serotypes. It remains unclear whether the buffalo can also act as carrier of the eurasian serotypes O and A, which occur in parts of East Africa. The screening of FMDV natural infection in buffalo and cattle was done using Enzyme Linked Immunosorbent Assay (ELISA), and then characterized by Solid phase competitive ELISA (SPCE) for FMDV antibodies specific to serotype O and A. The FMDV RNA screening and typing was done with one-step RT-PCR using PAN primers and serotype specific primers respectively. Results showed evidence for FMDV types O and A in buffalo and provides an important contribution to the knowledge for FMDV epidemiology in order to improve future tailored FMD control strategies in Tanzania.

Keywords: *Foot and mouth disease; Foot and mouth disease virus, livestock-wildlife interface areas, Serotype O, Serotype A, Tanzania.*

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious viral disease of even-toed domestic and wild ungulates caused by FMD *virus* (FMDV). It is a disease with potential food insecurity and socio-economic implications to the global community (Knight-Jones and Rushton, 2013). FMDV is a single stranded positive sense RNA virus classified into genus *Aphthovirus* of the family *Picornaviridae* (King, *et al.*, 2000; Zell *et al.*, 2017). The virion is non-enveloped with an icosahedral symmetry comprised of 60 copies of the structural viral proteins VP1, VP2, VP3 and VP4, with an estimated size of 30 nm in diameter (Knowles and Samuel, 2003). The virus exists in the form of seven antigenically distinct serotypes named as A, O, C, Asia1, South African Territories 1 (SAT1), SAT2, and SAT3. Of

the seven serotypes, Africa has identified and reported six serotypes (A, O, C, SAT1, SAT2 and SAT3). Except for Asia1 that has never been reported in Africa, serotypes O, A, SAT1 and 2 have been reported circulating and causing outbreaks of FMD in domestic animals in Tanzania (Kasanga *et al.*, 2015; Kasanga *et al.*, 2012; Sallu *et al.*, 2014; Vosloo *et al.*, 2002). The SAT 1-3 circulate as restricted to the African continent, and have occasionally been reported to cause outbreaks in the middle east countries (Jamal and Belsham, 2013). The FMDV type Asia1 occurs as restricted to Asia also and have rarely been reported to the western and eastern Eurasia whereas, the FMDV types O and A present an extended distribution, as are reported in Africa, Asia, and South America (Brito et al., 2017; Valarcher et al., 2009; Kitching et al., 2007). There are currently no reports for FMDV type C since 2004 (Brito et al., 2017; Sangula et al., 2011). These virus types circulate in seven conjectured epidemiological pools where pool 4 - 6 belong to Africa (Brito et al., 2017).

There are some pressing factors that make FMD control to be challenging and difficult to achieve in the foreseeable future one of them being its broad host spectrum. Literatures describe FMDV to be potentially infective to over 70 species of livestock and wildlife origin. In the livestock and wildlife animal populations susceptible to FMDV, cattle and buffalo have been identified most as the main species playing role in the transmission and as reservoirs of the virus respectively (Omondi et al., 2018). The large proportion of cattle herds are under pastoral communities, and these keep their animals in the proximity of conserved lands strategically for grazing their animals during pastures deprived seasons of the year (Bronsvoort *et al.*, 2004; Fè Vre *et al.*, 2006). Uncontrolled movements of animals increase interactions frequency between livestock and wildlife, sustain virus spread and transmissions, thereby complicating the epidemiology of the disease in the country or the entire region as well (Vosloo *et al.*, 2005). The complicated FMD epidemiological situation persisting in various geographic areas for decades, have enabled to the evolution of a large number of subtypes or topotypes within each serotype that portray significant genetic and antigenic distinct characteristics (Martínez, *et al.*, 1992).

Studies carried out in southern Africa for the persistently infected African buffalo have so far been potentially proved that, buffalo (*Syncerus Caffer*) successfully transmit FMDV serotypes SAT1, SAT 2 and SAT 3 to cattle (Thomson *et al.*, 2018). Persistently infected cattle and buffalo (*Syncerus Caffer*) are cited to be the potential sources of new FMD outbreaks in endemic countries (Grubman and Baxt, 2004), and they can maintain the virus for 6 month-3 years and 5-over 24 years respectively (OIE, 2009). In the persistently infected cattle and buffalo FMDV is maintained in Oesophageo-pharyngeal epithelial cells (Longjam *et al.*, 2011; Thomson, 1996). And the major means of viral transmission at interface is through animals interactions that occur either within conserved lands or at close vicinity communal grazing lands (Mkama *et al.*, 2014). It is not known whether African buffalo can also act as carriers for the Eurasian FMDV serotypes O and A as is the case in cattle. The present study investigated the serotypes O and A FMDV infection status of buffalo (*Syncerus Caffer*) and cattle in sera samples and probing samples from selected livestock-wildlife interface areas of Tanzania.

MATERIALS AND METHODS

The samples for this study were collected from cattle and buffalo in the select wildlife-livestock interface areas of Mikumi (Morogoro region), Katavi (Rukwa region), Ruaha (Iringa region), Mkomazi (Kilimanjaro region), and Serengeti (Mara and Manyara regions) national parks in Tanzania (Figure 1). The regions were selected on the basis of history of FMD outbreaks as described previously (Kasanga et al., 2012; Kivaria, 2003; Picado et al., 2011; Sallu et al., 2014).

Study design, sample type(s), and sampling strategy

This was a cross sectional study where by sera and esophageo-pharyngeal fluid or probang samples were analysed. At every interface area, sampling was done on buffalo herds then on cattle herds in their vicinity. The cattle herds in close proximity to buffalo were considered to have a greater likelihood of interacting to buffalo herds. In the field, the obtained sera samples were temporarily stored in labeled sterile cryovials in a cool box with icepacks (+4-6°C) and stored at -20ºC in the laboratory till when analyzed. Whereas, the obtained buffalo probang samples in sterile cryovials with viral transport media (VTM) were properly labeled and temporarily stored in a Liquid Nitrogen dry shipper until when the samples were transported to the laboratory and stored at -80ºC until use.

Screening for FMDV infection in buffalo and cattle

Screening of FMDV virus in cattle was done using PrioCHECK® FMDV NS antibody test ELISA kit, a non-species specific kit that detects antibodies directed against non-structural 3ABC proteins of FMDV (Clavijo *et al.*, 2004; Sorensen *et al.*, 1998;Mackay *et al.*, 1998). The test was done as per manufacturer's (PrioCHECK® FMDV NS, Prionics Lelystad B.V, Netherlands) instructions manual supplied with kit of Lot number: F120401L. Different tests are used for each of FMDV serotypes was done as previously described (Mackay *et al.*, 2001; Paiba *et al.*, 2004). The Solid phase competitive ELISA (SPCE) assay was deployed to characterize FMDV serotypes O and A. This assay was performed based on the manufacturer's (IZSLER Biotechnology Laboratory, Brescia, Italy) instructions manual supplied with kits of Lot number: 01-2012 120730b.

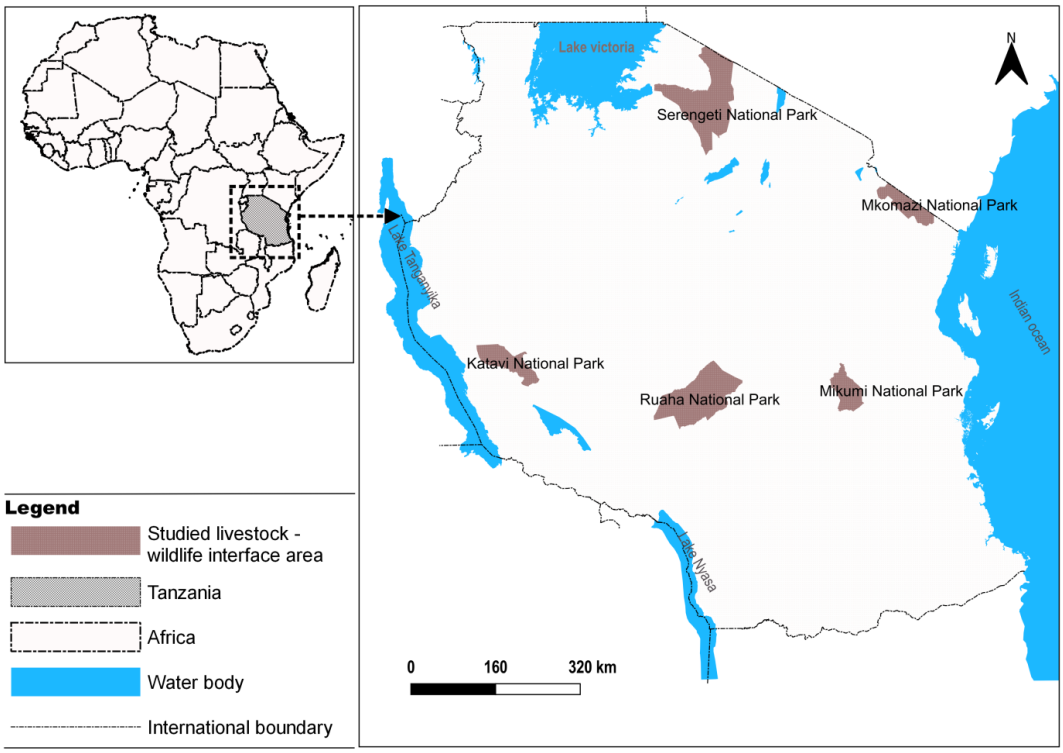

Figure 1. Map of Tanzania showing different livestock-wildlife interface areas where buffalo and cattle samples were obtained (Source; This study).

Detection and typing of FMDV type O and A

Samples were taken from -80^{0}C storage condition and allowed to equilibrate at room temperature, centrifuged at 12000 rpm and 500µl supernatant collected using 1000µl capacity pipette for RNA extraction. The RNA extraction was done using Qiagen RNeasy® Mini Kit by following the manufacturers' instructions manual, where 50µl RNA was obtained after elution. The extraction products were quantified spectrophotometrically using Nanodrop and all product below the ratio of 2.0 were rejected for further analysis. The genomic products obtained were screened to infer the presence of FMDV genomes in every field samples under study.

The screening was done by a one-step RT-PCR using PAN primers (Forward: GCCTGGTCTTTCCAGGTCT; Reverse: CCAGTCCCCTTCTCAGATC) that targets 5'UTR region of the FMDV genome. The protocol involved 50^{0}C (30 min.) for reverse transcription, 95^{0}C (15 min.) for (transcriptase enzyme denaturation, polymerase activation and cDNA unwinding), denaturation 95^{0}C (1 min.), annealing 55^{0}C (1 min.), elongation 72^{0}C (2 min.) and final elongation 72^{0}C (5 min.) for 35 cycles. The PCR amplicons generated were observed under a 1.5% Agarose gel electrophoresis and SafeView™ Classic ladder of 100bp size. The samples that tested positive for FMDV PAN- Primers were further analyzed by using FMDV serotypes (O & A) specific primers (FMDV type-O, Forward: CCTCCTTCAAYTACGGTG; Reverse: GCCACAATCTTYTGTTTGTG; Probe: [6FAM] CCCTCTTCATGCGGTARAGCAG[BHQ 1]; FMDV type-A, Forward: GCCACRACCATCCACGA; Reverse: GAAGGGCCCAGGGTTGGACTC; Probe: [6FAM] CTCGTGCGMATGAARCGGGC[BHQ1]) and the PCR amplification protocol was 50^{0}C (30 min.) for reverse transcription, 95^{0}C (15 min.) for (transcriptase enzyme denaturation, polymerase activation and cDNA unwinding), denaturation 95^{0}C (1 min.), annealing 60^{0}C (1 min.), elongation 72^{0}C (2 min.) for 35 cycles and final elongation 72^{0}C (5 min.) as described previously (Bachanek-Bankowska et al., 2016).

Data management and statistical analysis

The FMD percentage sero-positivity (PS) were calculated by dividing the total number of serotype specific (type O, A, or O&A) positive samples to SPCE test to the total number of non-structural protein ELISA (NSPEs) positive samples tested (Mwiine *et al.*, 2010). This study information was managed using Microsoft excel v.2013, and descriptive analyses, chi-square test, as well as independent t-test level were deployed to analyze and compare the PS variations for detected FMDV type O, A and O&A from cattle and buffalo sera samples plus their significance at 95% confidence.

RESULTS

In this study, a total of 247 3ABC-NSP ELISA positive serum samples from buffalo (n = 93) and cattle (n = 154) herds were serotyped by SPCE assay for detection of antibodies specific to FMDV serotype O and A and the SPCE results obtained were summarized in Table 1. The findings portrayed in Table 1 elaborate the different score levels for antibodies specific to FMDV type O and A on buffalo and cattle NSPE positive sample tested. The scores show the counts together with their corresponding percentages. The columns of type O&A mixed sero-reactions and that of the samples that did neither test positive for FMDV type O nor A were also included. Results from table 2 shows that out of the 93 analyzed NSPE positive buffalo samples, 30.1% (28) of CI (19 – 39) had antibodies specific to FMDV Type A. Also 54.8% (51) of CI (39 – 64) and 24.7% (23) of CI (15 – 33) had

antibodies specific to FMDV type O and those of mixed sero reaction respectively. Similarly, out of 154 NSPE positive cattle samples analyzed 53.9% (83) of CI (69 – 98), 66.9% (103) of CI (88 – 118) and 36.4% (56) of CI (44 – 70) had FMDV antibodies specific to type A, O and mixed sero-reactions respectively. The results for the molecular screening of probang samples obtained from clinically normal buffalos of the Serengeti national park showed that, 3 (3.37%) of the 89 probang sample extraction products indicated presence of FMDV genomic materials and when typed using FMDV serotype O and A specific primer, FMDV serotype O was revealed from only 1 sample (1.12%). Results are summarized in Figures 1-5, and amplification cure of RT-PCR for Srotype A and O specific primers (Figure 6).

Table 1. Serological characterisation of cattle and buffalo sera samples using SPCE for detection of antibodies specific to FMDV types O and A

National park	Animal Spp.	Samples Tested *(Mkama et al., 2014)*	NSPE +Ves	SPCE			Type-A&O -Ves(%)
				Type-A (%)	Type-O (%)	Type-A&O(%)	
Katavi	Cattle	61	49	27(55.1)	39(75.6)	21(42.9)	4(8.2)
	Buffalo	29	29	13(44.8)	25(86.2)	11(37.9)	4(13.8)
Ruaha	Cattle	53	41	13(31.7)	33(80.5)	11(26.8)	6(14.6)
	Buffalo	31	29	9(31.0)	20(69.0)	8(27.6)	9(31.0)
Mikumi	Cattle	35	29	17(58.6)	14(48.3)	11(37.9)	9(31.0)
	Buffalo	30	28	4(14.3)	5(17.9)	4(14.3)	23(82.1)
Mkomazi	Cattle	60	35	26(74.3)	17(48.6)	13(37.1)	5(14.3)
	Buffalo	31	7	2(28.6)	1(14.3)	1(14.3)	6(85.7)
Total	Cattle	209	154	83(53.9)	103(66.9)	56(36.4)	24(15.6)
	Buffalo	121	93	28(30.1)	51(54.8)	23(24.7)	42(45.2)
		330	247	106/247	154/247	79/247	66/247

Chi-square test of df=3, p <0.001; +Ves = positive samples; -Ves = negative samples

Table 2. Descriptive analysis of FMDV serotypes A and O in buffalo and cattle at selected livestock-wildlife interface areas in Tanzania

Species	FMDV serotypes	Frequency positives	%age	Standard Error	95.0% Lower CL	95.0% Upper CL
Buffalo	Type-A	28	30.10%	5	19	39
	Type-O	51	54.80%	6	39	64
	Type- A&O	23	24.70%	5	15	33
	Total	93	100.00%	8	79	108
Cattle	Type-A	83	53.90%	7	69	98
	Type-O	103	66.90%	8	88	118
	Type- A&O	56	36.40%	7	44	70
	Total	154	100.00%	8	139	168

Chi-square test of df=2, p <0.001

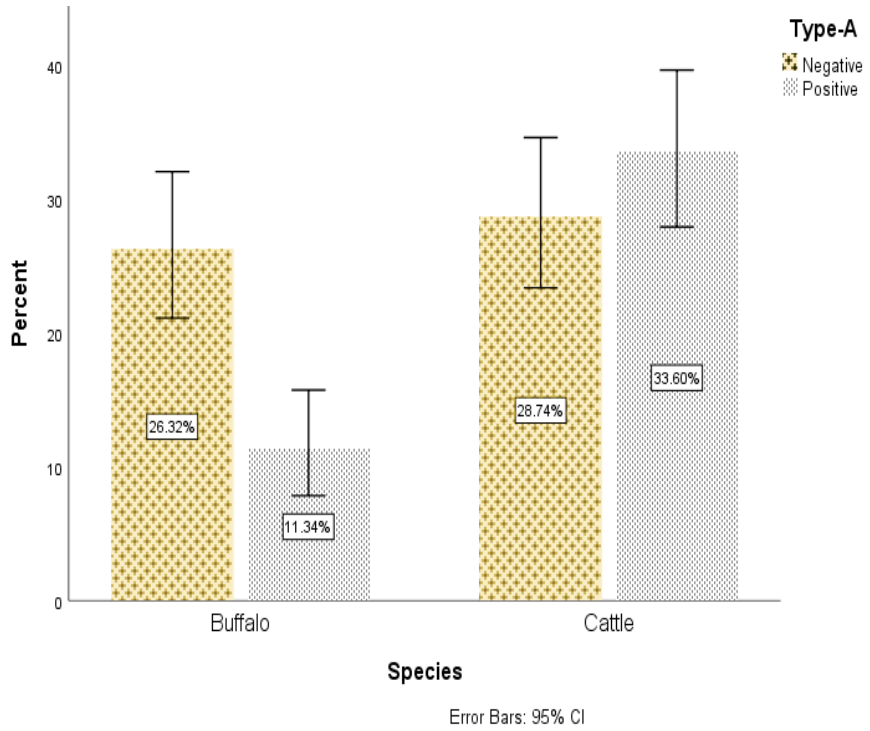

Figure 2. The overall infection rates for FMDV serotype A across buffalo and cattle at the studied livestock-wildlife interface areas in Tanzania.

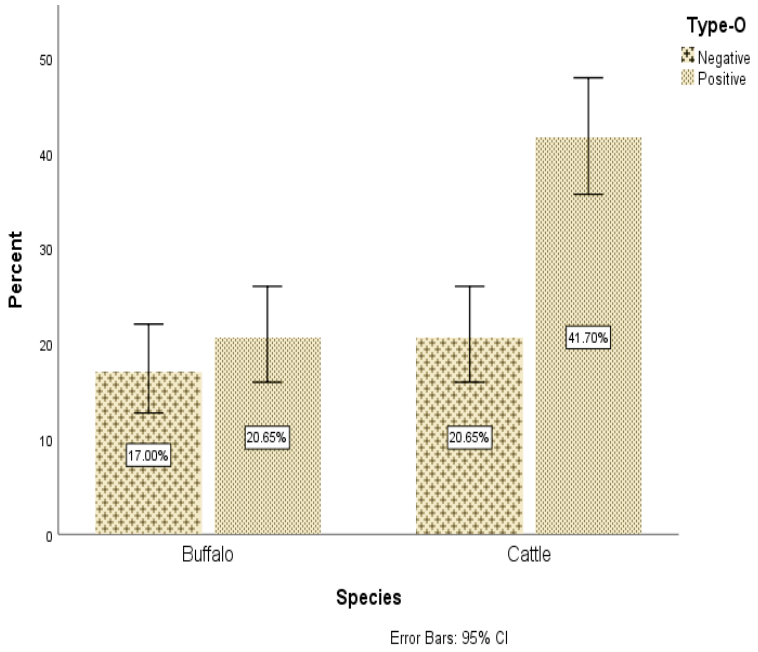

Figure 3. The overall infection rates for FMDV serotype O across buffalo and cattle at the studied livestock-wildlife interface areas in Tanzania.

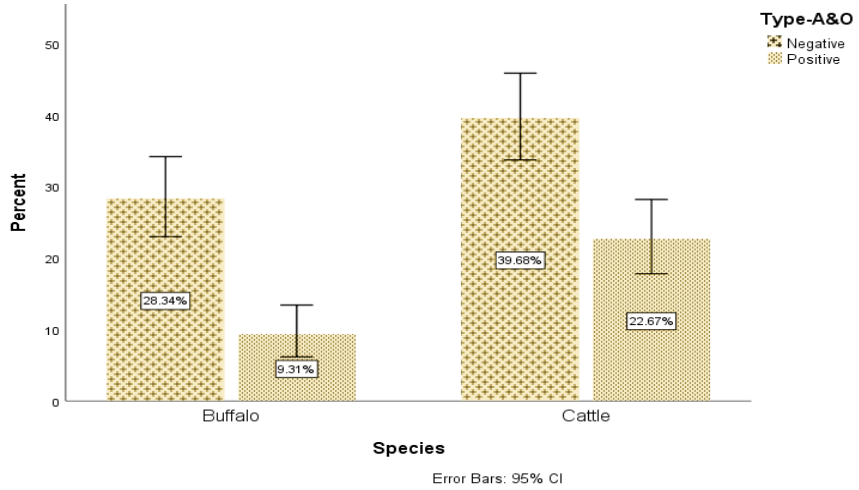

Figure 4. The overall FMDV serotypes O&A mixed infection across buffalo and cattle at the livestock-wildlife interface areas in Tanzania.

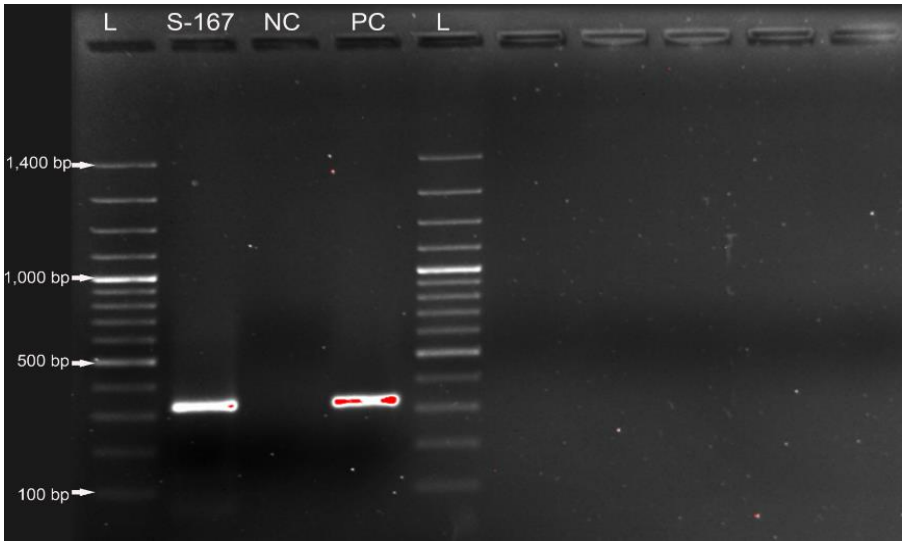

Figure 5. The agarose gel electrophoresis image showing PCR products at the expected 328bp band size after amplification using FMDV PAN-primers on buffalo probang sample S-167.

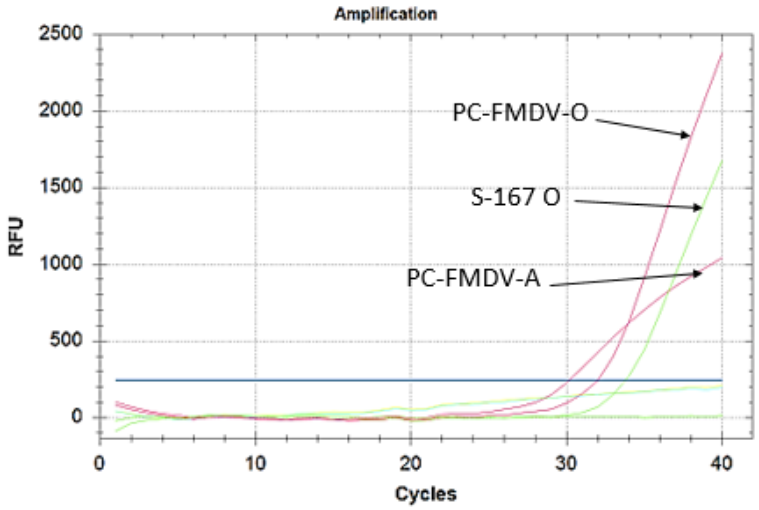

Figure 6: Molecular typing amplification curves derived from qRT-PCR assay using FMDV serotype O and A specific primer. The abbreviations PC-FMDV-O, PC-FMDV-A and S-167 means positive control for FMDV type O and A together with sample S-167 amplification curve positive for FMDV type O specific primers respectively.

DISCUSSION

Traditionally, the pastoral cattle herds are frequently grazed within or around national parks and intermingle with buffalo. The interactions between cattle and buffalo increases during drought periods or when most of the communal grazing areas get cultivated (Michael *et al.,* 2015), and contributes to the spread of pathogens as it is the case of FMDV (Thomson *et al.,* 2018). This study results shows FMDV to be prevalent in all livestock-wildlife interface areas studied. None of the sampled cattle and buffalo had a history of being vaccinated against any of the FMDV serotype(s) suggesting that, all sero-reactions resulted from FMDV natural infection. Furthermore, all sera samples from buffalo and cattle livestock-wildlife interface areas demonstrated the presence of both FMDV serotypes O and A at different percentage levels of sero-positivity (Figures 2–4).

The FMDV serotypes O or A detected in either buffalo or cattle sera samples, was similarly detected in the counter side herds of their vicinity. This suggests the co-occurrence and circulation of FMDV serotypes O and A between herds of cattle and buffalo in the field as it has been similarly stated in the Vosloo *et al.* (2002) study. An overall results shows a higher seropositivity (SP) of FMDV serotypes O and A in cattle than in buffalo at every livestock-wildlife interface area studied, except for Katavi interface (Table 1). The Katavi buffalo expressed a higher SP of 86% to FMDV serotype O than cattle in their vicinity, which showed a SP value of 76% (Table 1). The distinctive SP disparity expressed by Katavi interface buffalo could be attributed by probable extent of active virus activity at time of sampling (TAWIRI, 2019) that could facilitate a rapid spread of FMDV, and FMDV infections amongst buffalo herds. However, this suggestion is subject for further research to establish evidences.

The results also show FMDV serotypes O and A as predominant in the far southern and southern highlands (Katavi and Ruaha) and eastern and northern parts (Mikumi and Mkomazi) national parks of Tanzania (Table 1). The existence of serotypes O and A in livestock-wildlife interphase has been reported elsewhere in Maasai-Mara, Tsavo, and Meru ecosystems where FMDV types O, and A were detected by RT-qPCR from cattle tissue samples, and but not in buffalo (Wekesa et al., 2015). Similrly, studies in Uganda demonstrated existence of antibodies against FMDV and upon serotype-specific testing, FMDV serotypes O, SAT1, SAT2, and SAT3 were found (Ayebazibwe *et al.,* 2010; Ruhweza, 2014). However, the study did FMDV isolation and RT-qPCR, but could not detect the Eurasian FMDV RNA in buffalo apart from the already known types SAT1–3.

In the current study, sera samples that tested positive to NSPE, 66/247 (26.7%) of the samples did not test positive to FMDV serotypes O and A by SPCE (Table 1) suggesting that other FMDV serotypes (SAT1–3, Asia1 and C) apart from FMDV serotypes O and A were present in the samples. The small number of positives samples (Figure 5) and low CT value expressed in the type O amplification curve of about 33.79 (Figure 6) cannot be ascertained, but could be related to buffalo infection status or other factors.

Lastly, apart from the reports on SAT 1, SAT 2, SAT 3, O, and A, there has been no FMD outbreak in Tanzania caused by FMDV serotypes Asia1 or C (Bronsvoort, et al., 2006; Vosloo et al., 2002). Probably the observed findings of low infection rate statuses from Mikumi and Mkomazi livestock-wildlife interface areas could be due to FMDV SAT serotypes predominance, an aspect that had not been examined in this study.

ACKNOWLEDGEMENT

This study was funded by World Bank through WB-ACE II Grant PAD 1436 to the Southern Africa Center for Infectious Diseases Surveillance-Africa Centre of Excellence II Project (SACIDS-ACE II), Sokoine University of Agriculture (SUA), Africa Development Bank (ADB) through Southern Africa Development Community

Transboundary Animal Diseases (SADC-TADS) Project, The Wellcome Trust via the Intermediate Fellowship for Public Health Tropical Medicine (IFPHTM) Project. We also acknowledge the support of Greater Serengeti Ecosystem PPR Project under Global Challenges Research Fund support, Tanzania Wildlife Research Institute (TAWIRI), Tanzania National Parks Authority (TANAPA), and Tanzania Veterinary Laboratory Agency (TVLA) staffs for their technical support

CONFLICT OF INTEREST

The authors declare that they have no conflict or competing interests that may have influenced them in writing this article.

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