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Seroprevalence of foot-and-mouth disease virus in cattle herds raised in Maasai Mara ecosystem in Kenya

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ABSTRACT

A cross-sectional study was carried out to determine foot-and-mouth disease (FMD) seroprevalence and identify risk factors of exposure among cattle herds raised in three zones with different types of land use and progressively distant from the Maasai Mara National Reserve (MMNR) boundary. We selected five villages purposively; two in zone 1 (area < 20 km from the MMNR), another two in zone 2 (area between 20 − 40 km away from the MMNR) and one in zone 3 (area > 40 km away from the MMNR). A total of 1170 cattle sera were collected from 390 herds in all the zones and tested for antibodies against the non-structural proteins (NSPs) of FMD virus (FMDV) using two 3ABC-based Enzyme-Linked Immunosorbent Assay ELISA kits. All sera samples were also screened for serotype-specific antibodies using Solid Phase Competitive ELISA (SPCE) kits (IZSLER, Italy). We targeted FMDV serotypes A, O, South African Territory [SAT] 1 and SAT 2, known to be endemic in East Africa including Kenya. Data on putative risk factors for FMD seropositivity in cattle were collected using a questionnaire. The overall apparent animal-level FMD seroprevalence based on the parallel comparison of the two anti-NSPs ELISA kits was 83.8 % (95 % CI; 81.8–85.9), and differed significantly across zones. Zone 1 had a higher seroprevalence than zones 2 and 3 (χ^2 = 116.1, df = 2, p < 0.001). In decreasing order, the overall seroprevalences of FMDV serotypes A, SAT 2, O and SAT 1 were 26.3 % (95 % CI; 23.5–29.2), 21.4 % (95 % CI; 18.8–24.0), 21.2 % (95 % CI; 18.7–23.9) and 13.1 % (95 % CI; 11.1–15.3), respectively. The distribution of these serotypes differed significantly between zones (p < 0.05) except for SAT 2 serotype (χ^2 = 0.90, df = 2, p = 0.639). Both serotypes A and O were more prevalent in zones 1 and 2 than zone 3 while serotype SAT 1, was higher in zone 3 compared to other zones. The results of multivariable analyses identified animal sex (i.e., female), raising of cattle in zones 1 and 2 (areas < 40 km away from the MMNR); mixing of cattle from multiple herds at watering points, and pastoral husbandry practices, as significant predictors of animal-level FMD seropositivity. This study established that FMD seroprevalence declined with distance from the MMNR.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease that affects cloven-hoofed livestock and wildlife species including cattle, sheep, goats, pigs and African buffalo (*Syncerus caffer*) (Jamal and Belsham, 2013). The disease is a major challenge to livestock production in endemic areas as it causes significant production losses, including calf mortality, abortion and reduced milk yields (Knight-Jones and Rushton, 2013). It is caused by the FMD virus (FMDV) of the genus *Aphthovirus*, within the *Picornaviridae* family (Longjam et al., 2011). The virus occurs in seven serologically and genetically distinct serotypes, with serotypes A, O, SAT 1 and SAT 2 having been reported in cattle and buffalo populations in East Africa (Wekesa et al., 2015). Serotype O accounts for the majority of outbreaks in this region, followed in order by A, SAT 2 and SAT 1 (Wekesa et al., 2013; Namatovu et al., 2015). Multiple genetically-distinguishable topotypes or strains may also occur within each serotype because FMDV has a high mutation rate (Brito et al., 2017). Due to the antigenic diversity between serotypes, recovery from one serotype does not provide cross-protection (Bari et al., 2014).

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The FMDV genome consists of a single-stranded ribonucleic acid (ss RNA) approximately 8400 nucleotides long, which encodes a polypeptide that cleaves into several non-structural proteins (NSPs) and four structural proteins (SPs) (Jamal and Belsham, 2013). Testing for anti-NSP antibodies is widely used in both FMD endemic areas (Brocchi et al., 2006) and FMD-free countries (Barnett et al., 2015) to differentiate infected from vaccinated animals, while the detection of anti-SPs among NSP positive animals is used to determine the serotype responsible for the immune response (Namatovu et al., 2015).

There is limited knowledge about the epidemiology of FMD within livestock and wildlife in the Maasai Mara ecosystem. This region is home to many wildlife species, providing local communities with tourism-related revenue, alongside income from livestock farming (Bedelian and Ogutu, 2017). Recent studies in the area have shown that an increased human population, development of infrastructure and land privatization have led to major changes in land use (Løvschal et al., 2017; Veldhuis et al., 2019). Examples include the creation of wildlife conservancies in areas surrounding Maasai Mara National Reserve (MMNR) and increased mixed crop-livestock agriculture in areas further away from the MMNR (Nthiwa et al., 2019). While wildlife conservancies are utilized for both wildlife conservation and livestock production, this type of land use has many challenges including livestock depredation, competition for pasture and water (Mukeka et al., 2019), and increased transmission of infectious diseases partly due to intensified interactions between livestock and wildlife in areas close to MMNR than those more distant from the MMNR (Nthiwa et al., 2019).

This study used FMDV as a case study pathogen to investigate how different land use types affect its prevalence in cattle herds raised in the Maasai Mara ecosystem. In particular, we determined FMD seroprevalence in cattle across three zones progressively distant from the MMNR boundary and identified putative risk factors associated with exposure. We also determined FMDV serotypes circulating among cattle herds in the area. Our study provides data on the current serological situation of FMD in the area that can guide identification and use of appropriate vaccines.

2. Materials and methods

2.1. Study area

This study was performed in the Maasai Mara ecosystem in South Western Kenya (Fig. 1). This area is a biodiversity hotspot for diverse wildlife species including the African buffalo (*Syncerus caffer*), blue wildebeests (*Connochaetes taurinus*) and impala (*Aepyceros melampus*) (Ogutu et al., 2009), all known to transmit FMDV to livestock in Africa (Weaver et al., 2013). The southern part of the Maasai Mara ecosystem comprises the MMNR, a protected area that is confluent with the Serengeti National park in northern Tanzania. The immediate areas surrounding the MMNR have been converted into wildlife conservancies, where livestock farming also occurs (Bedelian and Ogutu, 2017). These territories are inhabited by Maasai pastoralists.

Three ecological zones with different land use types and progressively distant from the MMNR were selected. These zones included zone 1 (area < 20 km from the MMNR), zone 2 (area between 20–40 km from MNNR) and zone 3 (area > 40 Km from MNNR). Land use in the area varied across the targeted zones. In zone 1, for example, cattle herds were raised predominantly in extensive pastoral systems as they grazed in the surrounding wildlife conservancies and the MMNR (Bedelian and Ogutu, 2017), while in zone 2, cattle herds were raised in sedentary systems as they grazed behind fenced lands (Enström et al., 2017). Livestock production was carried out alongside crop agriculture in areas more distant from the MMNR (zone 3) (Bartzke et al., 2018). Given these types of land use, animals raised in areas close to the MMNR were therefore expected to have a higher relative risk of FMDV exposure than those raised in more distant areas.

2.2. Selection of villages

Preliminary participatory meetings were planned with the local communities to identify villages based on their degree of wildlife-livestock interactions. Five villages were selected purposively to represent the described zones above. In zone 1, we selected two villages (Mara Rianta and Oloolaimutia), another two in zone 2 (Lemek and Endoinyio Narasha) and one in zone 3 (Nkorinkori). Selected villages had typical characteristics of each zone as described above.

2.3. Study design, sample size estimation and epidemiological data collection

This study used a cross-sectional study with multistage cluster sampling. Data were collected between September 2016 and July 2017. We used the formula; $n = (1.96)^2 p(1-p)/d^2$, with a margin error (d) of 0.05 (Dohoo et al., 2012) to determine the number of animals (n) to sample in each zone. The study assumed a seroprevalence (p) of 50 % for FMD, given that seroprevalence information for this disease is limited in the area. We accounted for design effect due to herd-level clustering of cattle by adjusting the initial sample size using the formula; $n^1 = n(1 + \rho(m-1))$, where n^1 is the adjusted sample size, ρ the intra-cluster (within-herd) correlation coefficient (ICC), and m the number of animals to be sampled in each herd (Dohoo et al., 2012). The study assumed an ICC of 0.1 for FMD and sampled 3 animals (randomly-selected) in each herd. A total of 465 animals (from 155 herds) were to be sampled in each zone after adjustment for within-herd clustering. While information on livestock figures in the targeted zones is very limited, the sampling of cattle herds within zones was based on the probability proportional to the herd size. More cattle herds were sampled in zones 1 and 2 as these were thought to have more herds compared to zone 3. Specifically, we sampled 465 cattle each from zones 1 and 2, and 240 cattle (from 80 herds) in zone 3. In each village, we randomly selected livestock-keeping households from a household list prepared with the assistance of the area chiefs. In each selected household, we sampled the herd found in the village at the time of visit as households could own more than one herd. Animals aged above one year were targeted, as these animals interact with others from different herds or wildlife at watering points and/or during grazing (Nthiwa et al., 2019). The relative risk of FMDV exposure was therefore expected to be higher among older animals than calves as the latter are normally not taken for grazing but kept within the farm. Older animals also travelled long distances and thus they could be used more reliably for FMDV surveillance in the area.

In each sampled household, we collected epidemiological data on putative risk factors for transmission of FMD in cattle using a questionnaire. The information collected at animal-level included animal sex and age, while at herd-level, we recorded cattle herd size (the number of animals belonging to the household at the time of visit), whether there were any FMD infections in the past year, herd husbandry practices (sedentary or semi-nomadic pastoralism), sources of breeding bulls, grazing strategies, watering sources, vaccination status of each sampled herd and whether animals were purchased in the past year. More details about the questionnaire are provided in Supplementary material S1.

2.4. Blood sample collection and processing

We collected 10 ml jugular blood from each animal using plain vacutainer tubes labelled with unique barcodes. The samples were stored in cool boxes at +4 °C and at the end of each day they were transported to the Kenya Wildlife Service's (KWS) field laboratory facility in the MMNR. To extract sera, clotted blood samples were centrifuged at 3000*g* (gravitational force) for 6 min and the sera obtained aliquoted into two 2 ml uniquely barcoded cryovials. The aliquots were kept at −20 °C until further processing at the International Livestock

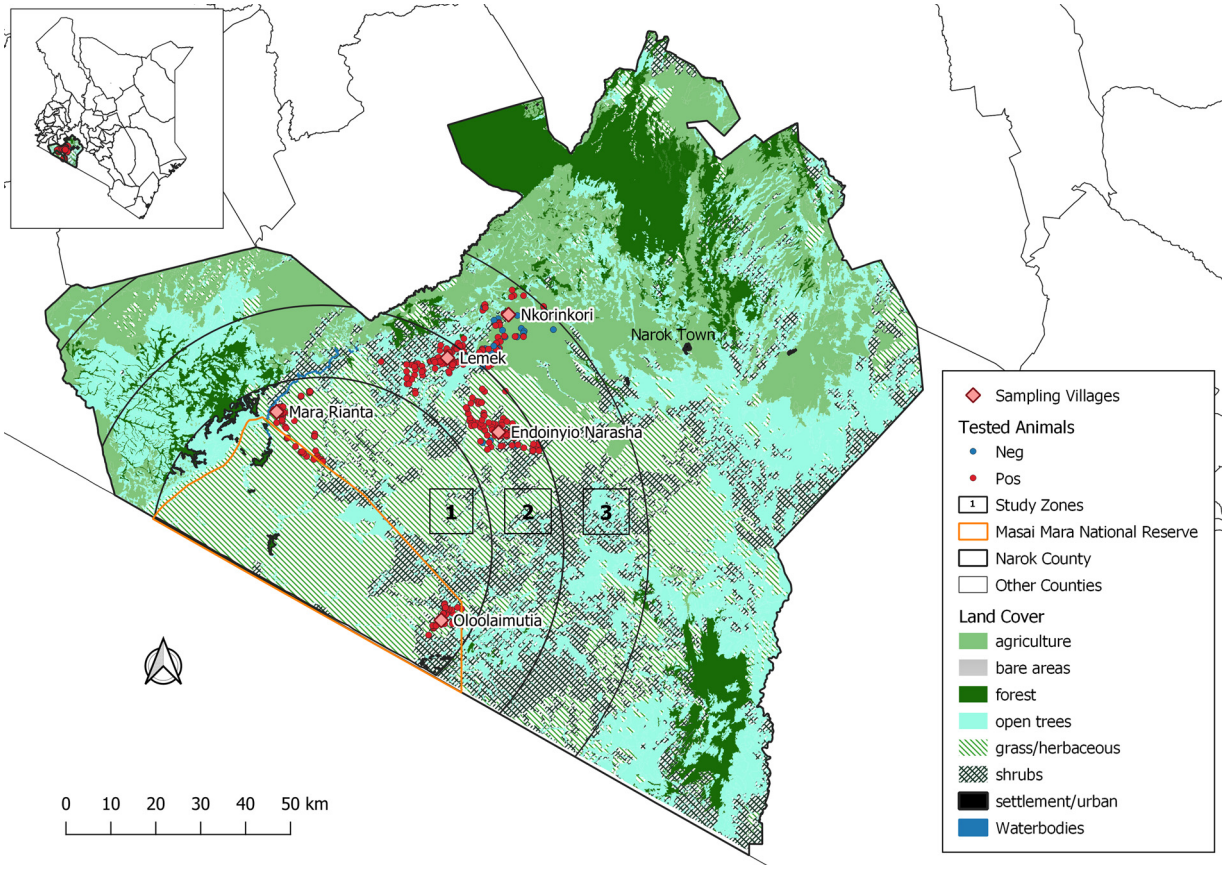

Fig. 1. Map showing sampling site locations and the distribution of NSP-seropositive animals within the surveyed zones.

Research Institute (ILRI) in Nairobi.

2.5. Serological testing

2.5.1. Detection of NSP antibodies against the FMDV

Sera were screened for antibodies against the non-structural 3ABC protein of FMDV using two NSP-based enzyme-linked immunosorbent assay (ELISA) tests. Initial screening was completed at ILRI using the PrioCHECK® FMDV NS blocking ELISA (Prionics, AG, Netherlands) following the manufacturer's instructions. Further screening was done at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), the OIE/FAO reference laboratory for FMD and swine vesicular disease in Brescia, Italy. For the initial screening completed at ILRI, optical densities (ODs) of samples and reference sera were measured at 450 nm using a microplate reader. The mean OD 450 of negative controls and the percentage inhibition (PI) of test sera for the PrioCHECK kit were calculated using the formula;

$$Percentage\ Inhibition\ (PI) = 100 - \left(\frac{OD\ 450\ of\ test\ sample}{mean\ OD\ 450\ of\ negative\ control}\right) \times 100$$

Samples were classified as negative if PI was < 50 % and positive if ≥ 50 %.

At IZSLER, sample screening was done using the previously validated IZSLER in-house 3ABC trapping indirect ELISA (Brocchi et al., 2006), in the format of ready-to-use kit (FMDV 3ABC-trapping ELISA, IZSLER, Brescia, Italy). In brief, the test sera, negative, weak positive and positive controls were run in duplicate wells. One well had 3ABC antigen trapped by a monoclonal antibody (MAb) while the other well contained MAb only. The ODs of test and reference sera were all read at 450 nm. To interpret the results, the net OD values of test and reference sera were calculated by subtracting the ODs of the wells without antigen from the corresponding ODs of the wells containing antigen. The percentage positivity (PP) of each test sample was then calculated as follows;

$$Percentage\ positivity\ (PP) = \frac{net\ OD\ value\ of\ test\ serum}{net\ OD\ value\ of\ positive\ control} \times 100\%$$

Animals were classified as negative if the PP was < 10 % and positive if PP was ≥ 10 %.

Both NSP-based ELISA kits were known to detect antibodies elicited by infection with any FMDV serotypes (Brocchi et al., 2006), including SAT 1 and SAT2 (Chitray et al., 2018). The diagnostic sensitivity and specificity of the two ELISA kits were, 86.4 % and 98.1 %, respectively, for PrioCHECK® FMDV NS ELISA kit and 86.4 % and 97.4 %, respectively, for IZSLER in-house 3ABC trapping ELISA (Brocchi et al., 2006).

2.5.2. Testing for serotype-specific antibodies against FMDV serotypes

All sera samples were also tested for serotype-specific antibodies (i.e., anti-FMDV structural proteins, SPs) using four ready-to-use MAb-based Solid Phase Competitive ELISA (SPCE) kits (IZSLER, Brescia, Italy) (Grazioli et al., 2008; Brocchi et al., 2012; Dho et al., 2014). The four serotype-specific ELISA kits targeted FMDV antibodies to serotypes O, A, SAT 1 and SAT 2 respectively, known to be endemic in East Africa (Wekesa et al., 2015). Briefly, sera were titrated for antibodies against these serotypes in three-fold serial dilutions from 1:10 to 1:270. Those with high antibody titres were re-tested at extended dilution to find the end-point antibody titre for each of the four FMDV serotypes tested. The end-point antibody titre was calculated as the reciprocal of the highest dilution producing 50 % inhibition.

2.6. Interpretation of serology results

We classified animals as seropositive if they tested positive to either

of the NSP ELISA tests, and negative if they tested negative to both tests. For the purposes of analysis, animals positive to the NSP test were considered infected, while animals negative to the NSP test were considered not-infected (Brocchi et al., 2006), regardless of the SP results. In the case of NSP-negative animals, if SP serology was positive, this was likely to be indicative of animals having been vaccinated, while SP-negative animals corresponded to unvaccinated and unexposed individuals (Longjam et al., 2011). In cases where sera from NSP-positive animals showed seropositivity to more than one serotype, we used differences in titres against serotypes of 3–4 folds to determine the serotypes responsible for the immune response. When SP-antibody titres against two or more serotypes were not significantly different, we considered animals as having been exposed to infection with multiple serotypes.

2.7. Ethical statement

This study obtained ethical and animal use approvals from the Institutional Research Ethics Committee (reference number ILRI-IREC 2016-02) and the animal care and use committee (reference number 2016–20) at ILRI. All interviewed farmers provided additional verbal consent for cattle blood sampling and the questionnaire survey

2.8. Data analysis

Results of serological analyses and the questionnaire data were entered into MS Excel (Microsoft® Excel, Washington, 2016) and imported into R software, version 3.6.0 (R Core Team, 2019) for analysis. Descriptive analyses such as the estimation of FMD seroprevalence with 95 % confidence interval being adjusted for herd-level clustering, were performed using the *epi.conf* function in *epiR* package (Stevenson et al., 2013). For the purposes of analysis, the main outcome (animal-level FMDV seropositivity) was defined by the results of the NSP tests interpreted in parallel (as above). The calculation of the true seroprevalence of FMD from the paralleled interpreted results of both NSP tests was performed using the *epi.prev* function in *epiR* package (Stevenson et al., 2013). The sensitivity (Se_p) and specificity (Sp_p) estimates of the paralleled compared results of both NSP tests used to calculate the true seroprevalence of FMD were estimated as follows;

$$Se_p = Se_1 + Se_2 - (Se_1 \times Se_2)$$

$$Sp_p = Sp_1 \times Sp_2$$

where Se_1 and Sp_1 were sensitivity and specificity estimates of the PrioCHECK® FMDV NS ELISA test, respectively, while Se_2 and Sp_2 denoted the sensitivity and specificity of the IZSLER in-house 3ABC trapping ELISA test, respectively (Dohoo et al., 2012). The Cohen's Kappa statistic was also used to estimate the level of agreement between the two NSP tests, while the χ^2 test was used to determine the association between categorical variables (animal sex and zone) and the animal-level seroprevalence of FMD.

Selected variables of interest were independently assessed for their association with the outcome using univariable logistic regression models. The analysis was done at animal-level and not at herd-level, given that the variability of FMDV exposure between herds was expected to be low. Directed Acyclic Graphs [DAGs] (Joffe et al., 2012) were then created for significant predictors ($p < 0.05$) in the univariable models to identify variables for multivariable logistic regression analysis. Both univariable and multivariable animal-level models were performed using generalized linear mixed-effects models (GLMM). We fitted data to these models using the *glmer* function in the *lme4* package (Bates et al., 2014) and accounted for herd-level clustering of cattle using herd ID as a random effect. The final multivariable logistic model was selected using a forward-backward stepwise procedure. We first fitted a saturated model with all significant predictors ($p < 0.05$) in the univariable models and retained those with $p < 0.05$ based on the Wald's χ^2 test. The final multivariable model was selected based on the lowest Alkaike Information Criterion (AIC). Two-factor product terms were created to assess the potential interaction effects of covariates in the final model. The statistical significance of the main effects of these two-factor interaction terms was determined using the likelihood ratio test (LRT). The final model's fit was evaluated by plotting the deviance residuals versus the fitted values obtained from the final model (Zhang, 2016). We also estimated the ICC for within-herd clustering of cattle using the *icc* function in *sjstats* package (Lüdecke, 2017). Sensitivity analysis was performed by comparing the results obtained using the paralleled interpreted NSP tests as the main outcome variable versus those of an alternative outcome variable comprising animals classified as seropositive if they had reactive antibodies to either NSP test, besides being SP-positive.

3. Results

3.1. Anti-NSP antibodies prevalence and distribution

In total 1,170 cattle sera (78.6% female and 21.4% male) from 390 herds were tested for antibodies against NSPs using two ELISA assays. The proportion of sampled herds described as vaccinated at the time of sampling was 44.9 %. The overall apparent animal-level and true seroprevalences of FMD were 83.8 % (95 % CI; 81.5–86.2) and 84.7 % (95 % CI; 82.4–86.9), respectively. The apparent animal-level seroprevalence of FMD differed significantly between locations where animals were kept. Zone 1 had a higher seroprevalence compared to zones 2 and 3 ($\chi^2 = 116.1$, df = 2, $p < 0.001$) (Table 1). The spatial distribution of NSP-positive animals in the surveyed zones is shown in Fig. 1. FMD animal-level seroprevalence also differed significantly by sex ($\chi^2 = 14.5$, df = 1, $p < 0.001$), with more female animals (86.0 %; 95 % CI; 83.8–88.2) being seropositive than males (76.0 %; 95 % CI; 70.8–81.2) (Table 1). The level of agreement between both ELISA tests was moderate (Cohen's Kappa statistic k = 0.6). The diagnostic sensitivity of both ELISA tests differed significantly (McNemar's $\chi^2 = 60.9$, df = 1, $p < 0.001$); the PrioCHECK® FMDV NS ELISA test detected more NSP positives, 81.2 % (95 % CI; 78.7–83.7) than the IZSLER in-house 3ABC trapping ELISA, 72.3 % (69.5–75.1).

3.2. Circulation of FMDV serotypes

Table 2 shows the results of the serotype-specific ELISA test (SPCE) and the distribution of circulating FMDV serotypes in the zones. A total of 49 (0.05 %) NSP positive serum samples were not included in the analysis because of undetectable SP antibodies in the SPCE ELISA tests, possibly connected with a faster SP antibody decline in these animals. In decreasing order, the overall seroprevalences of FMDV serotypes A, SAT 2, O and SAT 1 were 26.3 % (95 % CI; 23.5–29.2), 21.4 % (95 % CI; 18.8–24.0), 21.2 % (95 % CI; 18.7–23.9) and 13.1 % (95 % CI; 11.1–15.3), respectively. The distribution of these serotypes differed significantly between zones ($p < 0.05$) except for SAT 2 serotype ($\chi^2 = 0.90$, df = 2, $p = 0.639$). Both serotypes A and O were more prevalent in zones 1 and 2 than zone 3 while serotype SAT 1 was higher in zone 3 compared with other zones. The estimated percentage of animals exposed to multiple serotypes was 18.0 % (95 % CI; 15.7–20.5) (Table 2). Across zones, there was a statistically significant difference in the proportion of animals showing serotype co-exposure ($\chi^2 = 8.16$, df = 2, $p = 0.017$).

3.3. Risk factors associated with animal-level FMD seropositivity

Table 1 shows predictors found in univariable analysis to be statistically significantly associated with animal-level seroprevalence of FMD (with adjustment for herd-level clustering). The results of the multivariable model identified animal sex (i.e., female), raising of cattle in areas close to MMNR (zones 1 and 2), mixing of cattle from multiple

Table 1
Variables associated with animal-level seroprevalence of FMD based on univariable logistic regression with random effect for herd ID.

Variable and category		FMD		
	No. tested (n)	% NSP prevalence (95 % CI)	Odds ratio (95 % CI)	P-value
Sex				
Male	250	76.0 (70.1–81.8)	1 (Ref.)	
Female	920	86.0 (83.5–88.4)	2.9 (1.8–4.7)	< 0.001
Zones*				
Zone 3	240	63.3 (57.5–69.9)	1 (Ref.)	
Zone 2	465	83.4 (79.7–87.2)	3.6 (2.2–5.9)	< 0.001
Zone 1	465	94.8 (92.6–97.0)	14.7 (7.8–27.7)	< 0.001
Shared watering sources within the village				
No	42	59.5 (42.6–76.5)	1 (Ref)	
Yes	1128	84.8 (82.5–87.1)	5.8 (1.9–17.6)	0.002
Shared watering points between villages				
No	510	75.3 (71.2–79.4)	1 (Ref)	
Yes	660	90.5 (88.0–92.9)	4.0 (2.5–6.5)	< 0.001
Contact with cattle from different herd at watering points				
No	291	70.1 (64.3–75.9)	1 (Ref)	
Yes	879	88.6 (86.1–90.7)	4.4 (2.7–7.4)	< 0.001
Contact with cattle from different herd during grazing				
No	177	71.8 (64.4–79.1)		
Yes	993	86.0 (83.6–88.4)	3.3 (1.8–6.0)	< 0.001
Grazing animals on pastures shared within village				
No	150	67.3 (59.1–75.6)	1 (Ref)	
Yes	1020	86.3 (84.0–89.0)	4.4 (2.3–8.3)	< 0.001
Grazing animals on pastures shared between villages				
No	873	80.0 (77.2–83.0)	1 (Ref)	
Yes	297	94.9 (92.2–97.7)	6.4 (3.1–13.1)	< 0 .001
Herd management practice				
Sedentary	660	75.5 (71.8–79.1)	1 (Ref.)	
Pastoral	510	94.7 (90.7–95.6)	7.6 (4.4–13.2)	< 0 .001
Grazing of cattle in wildlife reserves				
No	666	76.4 (72.9–80.0)	1 (Ref.)	
Yes	504	93.7 (91.3–96.0)	6.2 (3.6–10.6)	< 0 .001

Ref, reference category; CI, confidence intervals.
*Zone 1 (area < 20 km from the MMNR).
Zone 2 (area between 20 − 40 km away from the MMNR).
Zone 3 (area > 40 km away from the MMNR).

herds at watering points and pastoral husbandry practices as significant predictors of animal-level FMD seropositivity (Table 3). The estimated ICC was 0.24 (95 % CI; 0.09–0.37) based on the variance components of the final multivariable model. The two-factor interaction terms of covariates in the final multivariable model did not show significant interaction effects ($p > 0.05$). The results from the sensitivity analysis (data not presented) were comparable to those of standard analysis based on the main outcome variable.

4. Discussion

This study determined the current serological status of FMD among cattle herds raised in three zones with different types of land use and suspected to be exposed to different levels of interactions with FMD-infected wild ungulates on the basis of their distance from the MMNR boundary. The high FMD seroprevalence reported by this study indicated that this disease is prevalent in the area, consistent with earlier studies (Onono et al., 2013; Nthiwa et al., 2019). We found a higher overall FMD seroprevalence than the national mean prevalence of 52.5 % (Kibore et al., 2013). The high exposure levels of FMDV in cattle could have significant implications on livestock production and trade, food security and livelihoods of the households that depend on livestock (Knight-Jones and Rushton, 2013). Control of FMD is also challenging as the disease is highly infectious and exposed animals could become persistently-infected, excreting low quantities of FMDV for several months (Arzt et al., 2018). It is estimated that about 50 % of

Table 2
Distribution and seroprevalence of FMDV serotypes in study zones.

FMDV serotypes	Zones* number of SP positive animals, percent (%) seroprevalence (95% CI)							
	Zone 3		Zone 2		Zone 1		Row total	
SAT 1	36	26.7 (20.0–34.6)	40	11.0 (8.0–14.0)	46	10.6 (8.1–13.6)	122	13.1 (11.1–15.3)
SAT 2	26	19.3 (23.3–26.1)	75	20.6 (16.6–24.9)	98	22.6 (18.9–26.7)	199	21.4 (18.8–24.0)
O	17	12.6 (8.1–18.5)	79	21.7 (17.6–25.9)	102	23.6 (19.6–27.6)	198	21.2 (18.7–23.9)
A	20	14.8 (9.6–20.9)	112	30.8 (26.1–35.7)	113	26.1 (22.2–30.4)	245	26.3 (23.5–29.2)
FMDV multiple serotypes (≥2)	36	26.7 (20.0–34.6)	58	15.9 (12.4–19.6)	74	17.1 (13.9–20.8)	168	18.0 (15.7–20.5)
Column total	135	14.5 (12.3–16.7)	364	39.1 (35.8–42.3)	433	46.5 (43.1–49.8)	932	

CI, confidence intervals.
*Zone 1 (area < 20 km from the MMNR).
Zone 2 (area between 20 − 40 km away from the MMNR).
Zone 3 (area > 40 km away from the MMNR).

Table 3
Final multivariable model of animal-level risk factors for FMD in cattle based on GLMM analysis with a random effect for herd ID.

Variables	Category	Odds ratio (95 % CI)	P-*value*
Fixed effects			
Animal sex			
	Male	1 (Ref.)	
	Female	4.0 (2.5–6.4)	< 0.001
Study zones*			
	Zone 3	1 (Ref.)	
	Zone 2	3.2 (1.9–5.5)	< 0.001
	Zone 1	6.6 (2.2–19.6)	0.001
Contact with cattle (from a different herd) at watering points			
	No	1 (Ref.)	
	Yes	1.7 (1.0–2.8)	0.045
Herd management practice			
	Sedentary	1 (Ref.)	
	Pastoral	2.6 (1.0–6.8)	0.049

Ref, reference category; CI, lower and upper limits for 95 % confidence intervals.
The random variable (i.e., herd ID) used to account for the within-herd clustering of FMD was 1.1 (95 % CI; 0.6–1.4).
*Zone 1 (area < 20 km from the MMNR).
Zone 2 (area between 20 − 40 km away from the MMNR).
Zone 3 (area > 40 km away from the MMNR).

exposed animals become persistently-infected irrespective of their vaccination status (Barnett et al., 2015). While the transmission risk of FMDV from carrier animals to susceptible animals in herds is low, it is still poorly understood (Arzt et al., 2018). Their existence in cattle populations can prevent farmers from accessing international markets for live animals and/or animal-source products (Knight-Jones and Rushton, 2013).

The estimated ICC of 0.24 reported by this study was moderate, although studies elsewhere have reported higher estimates, for example, 0.360-0.553 in Switzerland (Kuster et al., 2015) and 0.36 in Iran (Emami et al., 2015). This finding indicates that FMDV exposure levels among animals within herds are correlated. The disease is highly infectious and multiple animals within a herd become infected at any one time.

This study found a significant association between FMD seroprevalence and animals' sex, with more females being exposed than males. In the Maasai Mara ecosystem, female animals have lower offtake rates compared with males, as they are kept for milk production and breeding purposes (Huho et al., 2011). Because females tend to stay in the herd for longer, they probably have a higher chance of FMDV exposure or repeated exposure to different FMDV serotypes or strains (Mesfinie et al., 2019).

This study found that FMD seroprevalence increased as sites got closer to the MMNR. This finding could be partly due to more frequent and intense interactions between cattle and FMD-infected wild ungulates in areas close to MMNR than those progressively distant from the MMNR boundary. However, we could not determine the role of wildlife in the observed seroprevalence as wildlife sampling was not conducted. This study also found that serotypes A, O, SAT 1 and SAT 2 were circulating in cattle, consistent with previous findings in the area (Bronsvoort et al., 2008; Wekesa et al., 2015). The seroprevalence of SAT 1 was highest in zone 3, while SAT 2 seroprevalence did not differ significantly between zones. Recent studies in the area (Wekesa et al., 2015) and in East Africa (Casey-Bryars et al., 2018; Omondi et al., 2019) have reported genetically distinct SAT 1 and 2 isolates circulating in sympatric cattle and buffalo populations and suggests that there could be genetic evolution of new FMDV strains in both populations. However, in East Africa, SAT 1 and SAT 2 circulate and are maintained in livestock populations independently from wildlife, while in Southern Africa, they are maintained exclusively in wildlife (Casey et al., 2014). It is therefore likely that the higher seroprevalence of SAT 1 in zone 3 compared to other zones could be partly due to livestock-related factors such as intra- and inter-herd contacts. For example, it is common within zone 3 for multiple herds to graze on fields of post-harvest maize and wheat straws, potentially increasing the transmission levels of this serotype through increased animal contact rates.

The different types of land use adopted in the selected zones is also a possible factor that may explain the higher seroprevalence of FMD in areas close to MMNR (zone 1) than in zones 2 and 3. Land use change is driven by the need to meet demand for food, timber, fibre, water and other resources in many parts of the world (Mastel et al., 2018). It is a major cause of environmental change (Patz et al., 2008), and can influence the transmission dynamics of infectious diseases through various mechanisms (Gottdenker et al., 2014). For example, land use change can either inhibit or promote interactions between host species (Hassell et al., 2017). These interactions (direct or indirect) can affect the level of microbe transmission between host species (Miguel et al., 2013). For example, in zone 1, cattle herds were grazed in MMNR and wildlife conservancies in predominantly pastoral systems. This type of livestock production involves moving the herd in search of water and pasture, which may increase the chances of susceptible cattle coming into contact with infected animals. In general, animal movements play a significant role in the spread of infectious diseases (Fèvre et al., 2006). Pastoral livestock production systems also involve utilization of grazing areas and watering sources by multiple herds, which can lead to close interactions between animals, and/or contamination of fomites at these shared sites (Ayebazibwe et al., 2010). Indeed, this study identified pastoral herd husbandry practice, sharing of grazing area, and watering sources as significant predictors of FMD seropositivity in cattle.

This study also found that some NSP-positive animals had serotype-reactive antibodies against more than one FMDV serotype. Pressure for co-exposure might be expected to be lower in zone 3, where NSP seroprevalence (virus circulation) is lower and distance from MMNR is increased. However, we found significantly higher levels of co-exposure in zone 3 than other zones. This could be due to cross-reactivity between serotypes that have common epitopes (Bari et al., 2014), or a heterotypic serological response arising from primary effects of previous exposure to one or multiple serotypes or vaccination (Namatovu et al., 2015). Nevertheless, it is also possible that these animals had been exposed to two or more serotypes, as previous studies in East Africa and elsewhere have shown that serotype predominance varies over time exposing cattle to different serotypes (Casey-Bryars et al., 2018; Ouagal et al., 2018).

This study had several limitations, including the use of serological tests to determine FMD seroprevalence and serotypes circulating in the area. The detection of anti-NSPs to infer infection or exposure may also be imperfect, as animals were classified as either positive or negative based on a cut-off threshold value. Vaccinated and subsequently infected animals with little or no systemic infection may also elicit a non-detectable anti-NSP immune response (Brocchi et al., 2006). In contrast, animals vaccinated with non-purified vaccines may seroconvert to NSPs (Lyons et al., 2015a). Furthermore, the co-existence of FMD infections and vaccinations in the Maasai Mara ecosystem could also complicate the interpretation of serological data (Knight-Jones et al., 2016), since a larger proportion (44.9 %) of the sampled herds had been vaccinated prior to sampling. The FMD vaccines used in Kenya are either monovalent or multivalent, but currently a quadrivalent vaccine (i.e., purified oil-based Fotivax ™) containing FMDV strains of serotypes O, A, SAT 1 and SAT 2 is being introduced (Lyons et al., 2015b). The testing for anti-SP antibodies to determine FMDV serotypes in NSP-positive animals is also limited by cross-reactions between serotypes, which is a common feature of ELISA assays (Morris et al., 2018). The level of cross-reactivity varies according to different immune responses of individual animals tested and different possible immune statuses (for example; vaccinated, infected, vaccinated and infected or vice versa,

and those infected by multiple serotypes). Additionally the cross-sectional study design used was unable to indicate which serotype(s) resulted in frequent outbreaks within the study area. While this study did not quantify livestock movements between the different zones at the time of sampling due to logistical constraints, a previous study conducted in the same area showed that livestock movements may be more prevalent during the dry season compared with the wet season, and are also dependent on the cattle herd size (Butt, 2010). The selected zones were contiguous; this may have influenced the results as it was not controlled for within the study design. Besides, the variable representing zones was entered as a fixed effect during the multivariable analysis rather than as a random variable.

5. Conclusions

Our study showed higher FMD seroprevalence in areas close to the MMNR than in areas more distant from the MMNR boundary. This difference could be partially explained by the influence of interactions between livestock and FMD-infected wild ungulates, which are likely to be higher in zone 1 and progressively lower in zones 2 and 3. We also found serotypes A, O, SAT 1 and SAT 2 circulating in cattle. Serotypes A and O were more prevalent in zones 1 and 2; whereas SAT 1 was highest in zone 3 compared with the other zones. The distribution of serotype SAT 2 did not vary significantly across zones. Vaccines used within this region should therefore include all four serotypes, matched with circulating virus strains for improved efficacy. Vaccination, using multivalent vaccines, should be intensified in cattle populations closest to the MMNR. Establishment of an FMD notification and surveillance system in the area is also required to ensure early case detection and timely outbreak management. Future studies should also quantify how wildlife-livestock interactions and livestock movements may influence FMD incidence in the area. The sampling of wildlife species would also provide useful information on the genetic diversity of FMDV in the area.

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Data availability

All relevant data are within the paper and its supporting information files.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2020.104929.

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