

**EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE,
BRUCELLOSIS AND LEPTOSPIROSIS AT THE LIVESTOCK-
WILDLIFE INTERFACE AREA IN MAASAI MARA, KENYA**

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REQUIREMENTS FOR THE AWARD OF THE DEGREE OF
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DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree or any other award.

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DEDICATION

This work is dedicated to my dear wife, Caroline who has been a constant source of encouragement and support during the course of my studies and in my life. I truly thank you for being there for me throughout the entire academic journey.

This thesis is also dedicated to my loving parents, the late Joseph and Josephine for the many ways you have contributed in my life. Thank you for supporting my determination to achieve my dreams.

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TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF PUBLICATIONS RELATED TO THIS THESIS	xi
LIST OF PRESENTATIONS RELATED TO THIS THESIS	xii
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF APPENDICES	xvi
LIST OF ABBREVIATIONS/ACRONYMS	xvii
ABSTRACT	xviii
CHAPTER ONE	1
1.0 INTRODUCTION	1
1.1 Background	1
1.2 Problem statement	3
1.3 Justification and significance of the study	3
1.4 Research questions	4
1.5 Hypotheses	5
1.6 Objectives	6
1.6.1 Overall objective	6

1.6.2 Specific objectives	6
CHAPTER TWO	7
2.0 LITERATURE REVIEW	7
2.1 Effects of land use changes on the transmission dynamics of infectious diseases.....	7
2.2 Aetiology and diagnosis of brucellosis, leptospirosis and FMD	9
2.2.1 Brucellosis.....	9
2.2.2 Leptospirosis	11
2.2.3. Foot and mouth disease (FMD)	12
2.2.3.1 Diagnosis of FMD using molecular-based tools	14
2.2.3.2 Discriminating between infected from vaccinated animals by ELISA	16
2.2.3.3 FMDV Serotype determination by ELISA methods	17
2.2.3.4 Pen-site FMD diagnostic approaches	17
CHAPTER THREE.....	19
3.0 KNOWLEDGE, ATTITUDES AND PRACTICES OF THE MAASAI PASTORALISTS ON MAJOR CATTLE DISEASES IN DIFFERENT ZONES OF MARA ECOSYSTEM WITH VARYING LEVELS OF WILDLIFE-LIVESTOCK INTERACTIONS	19
3.1 Abstract	19
3.2 Introduction.....	20
3.3 Materials and methods	21
3.3.1 Study area.....	21
3.3.2 Compliance with ethical standards	23

3.3.3 Study design and selection of villages	24
3.3.4 Data collection	24
3.3.5 Herd species composition	25
3.3.6 Livestock benefits and disease constraints to cattle production	26
3.3.7 FMD annual age-specific prevalence and mortality	27
3.3.8 Impacts of livestock diseases on livelihoods from livestock.....	27
3.3.9 Data management and analysis.....	28
3.4 Results	29
3.4.1 Livestock species	29
3.4.2 Ranking of benefits from livestock.....	30
3.4.3 Prioritization of livestock diseases	33
3.4.4 FMD prevalence, mortality and impacts on market value.....	34
3.4.5 Disease impact matrix scoring results	35
3.5 Discussion	38
3.6 Conclusion.....	41
CHAPTER FOUR.....	43
4.0 SEROPREVALENCE AND RISK FACTORS OF <i>LEPTOSPIRA</i> SPP. AND <i>BRUCELLA</i> SPP. IN CATTLE AT THE WILDLIFE-LIVESTOCK INTERFACE AREA IN MAASAI MARA, KENYA.....	43
4.1 Abstract	43
4.2 Introduction.....	44
4.3 Materials and methods	45
4.3.1 Study area.....	45

4.3.2 Selection of villages.....	46
4.3.3 Study design, sample size estimation and collection of epidemiological data	47
4.3.4 Sample collection and processing.....	49
4.3.5 Serological testing.....	49
4.3.5.1 <i>Brucella</i> spp. antibody test	49
4.3.5.2 <i>Leptospira</i> spp. antibody test.....	50
4.3.6 Data analyses	50
4.4 Results	52
4.4.1 Descriptive results.....	52
4.4.2 Risk factors associated with <i>Brucella</i> spp. seropositivity	55
4.4.3 Risk factors associated with <i>Leptospira</i> spp. seropositivity.....	63
4.5 Discussion	64
4.6 Limitations	69
4.7 Conclusion.....	70
CHAPTER FIVE.....	71
5.0 SEROPREVALENCE AND RISK FACTORS OF FOOT AND MOUTH DISEASE VIRUS IN CATTLE AT THE WILDLIFE- LIVESTOCK INTERFACE AREA IN MAASAI MARA, KENYA	71
5.1 Abstract	71
5.2 Introduction.....	72
5.3 Materials and methods	74
5.3.1 Study site and sampling framework.....	74

5.3.2 Serological testing.....	74
5.3.2.1 Detection of NSP antibodies against the FMD virus.....	74
5.3.2.2 Testing for serotype-specific antibodies against FMDV serotypes	76
5.3.2.3 Interpretation of serology results	76
5.3.3 Data analysis	77
5.4 Results	79
5.4.1 Anti-NSP antibodies prevalence and distribution.....	79
5.4.2 Circulation of FMDV serotypes	81
5.4.3 Risk factors associated with animal-level FMD seropositivity	84
5.5 Discussion	85
5.6 Limitations	89
5.7 Conclusions	91
CHAPTER SIX.....	92
6.0 MODELLING VACCINATION STRATEGIES AGAINST FOOT AND MOUTH DISEASE IN LIVESTOCK IN MAASAI MARA, KENYA	92
6.1 Abstract	92
6.2 Introduction.....	93
6.3 Materials and methods	95
6.3.1 Model formulation and description.....	95
6.3.2 Building of a baseline model	99
6.3.3 Vaccination scenario analyses	100

6.4 Results	101
6.4.1 Cumulative incidence of FMD in cattle and sheep populations....	101
6.4.2 Results of vaccination scenario analyses	102
6.5 Discussion	107
6.6 Limitations	109
6.7 Conclusions	110
CHAPTER SEVEN.....	111
7.0 CONCLUSION AND RECOMMENDATIONS	111
7.1 Conclusion.....	111
7.2 Recommendations.....	113
REFERENCES.....	115
APPENDICES.....	131

LIST OF PUBLICATIONS RELATED TO THIS THESIS

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2. **Nthiwa, D.**, Alonso, S., Odongo, D., Kenya, E. & Bett, B. (2019). A participatory epidemiological study of major cattle diseases amongst Maasai pastoralists living in wildlife-livestock interfaces, Maasai Mara, Kenya. *Tropical Animal Health and Production*, 51 (5): 1097-1103. <https://doi.org/10.1007/s11250-018-01790-1>
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6. **Nthiwa, D.**, Alonso, S., Odongo, D., Kenya, E. and Bett, B. 2017. Participatory epidemiology of foot and mouth disease amongst the Maasai pastoralists living in wildlife-livestock interfaces, Maasai Mara, Kenya. **Oral presentation** at the 1st annual postgraduate seminar, University of Embu, 25th May 2017. Book of abstracts page 22/30.

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LIST OF TABLES

Table 3.1: Median scores and their respective ranges obtained from ranking livestock species kept by the Maasai in the Mara ecosystem, Kenya.	30
Table 3.2: Overall relative importance of livestock benefits, results obtained by proportional piling.....	32
Table 3.3: Overall summarized disease impact matrix scoring results.....	37
Table 4.1: Animal- and herd-level apparent seroprevalences of <i>Brucella</i> spp. and <i>Leptospira</i> spp. and the levels of co-exposure in various zones	54
Table 4.2: Risk factors associated with animal-level seroprevalence of <i>Brucella</i> spp. and <i>Leptospira</i> spp. based on univariable logistic regression with a random effect for herd	57
Table 4.3: Risk factors associated with herd-level seroprevalence of <i>Brucella</i> spp. and <i>Leptospira</i> spp. based on univariable logistic regression with a random effect for village.	59
Table 4.4: Final models of animal-level risk factors for <i>Brucella</i> spp. and <i>Leptospira</i> spp. in cattle based on GLMM analysis.....	61
Table 4.5: Final models of herd-level risk factors for <i>Brucella</i> spp. and <i>Leptospira</i> spp. in cattle GLMM analysis.....	62
Table 5.1: Variables associated with animal-level seroprevalence of FMD based on univariable logistic regression with random effect for herd	80
Table 5.2: Distribution and seroprevalence of FMDV serotypes in various zones	83
Table 5.3: Final multivariable model of animal-level risk factors for FMD in cattle based on GLMM analysis with a random effect for herd.....	85
Table 6.1: Literature biological parameters of FMDV used in the model	98
Table 6.2: Reduced cumulative incidence of FMD in cattle and sheep host species under different vaccination strategies and coverages.....	107

LIST OF FIGURES

Figure 3.1: Map of Kenya showing the Maasai Mara ecosystem and the locations of the survey sites.	23
Figure 4.1: Location of sampling sites within the Maasai Mara ecosystem	47
Figure 4.2: Spatial distribution of <i>Brucella</i> spp. (in blue dots) and <i>Leptospira</i> spp. (in red dots) seropositive herds in various ecological zones with different levels of wildlife-livestock interactions.	55
Figure 5.1: Map of Maasai Mara ecosystem showing the spatial distribution of NSP seropositive animals in the surveyed zones.....	81
Figure 6.1: Model flow diagram showing the SEIR compartments in boxes and the transitions of animals between compartments by arrows.....	97
Figure 6.2: Predicted FMD incidence in cattle and sheep populations in the first 150 days of the outbreak.	102
Figure 6.3: Estimated incidence of FMD averted in cattle [a] and sheep [b] by vaccinating cattle alone on day 1 of the outbreak compared to baseline model. Simulated incidence in the first 100 days of the outbreak.	104
Figure 6.4: Estimated incidence of FMD averted in cattle [a] and sheep [b] by vaccinating both host species on day 1 of the outbreak compared to baseline model. Simulated incidence in the first 100 days of the outbreak	105

LIST OF APPENDICES

Appendix 1: ILRI Institutional Research Ethical approval.....	131
Appendix 2: ILRI Institutional Animal Care and Use Committee approval	132
Appendix 3: Questionnaire	134
Appendix: 4: Difference equations used to model the transmission of FMD virus between cattle and sheep.....	139

LIST OF ABBREVIATIONS/ACRONYMS

AIC	: Akaike Information Criterion
A4NH	: Agriculture for Nutrition and Health
AAT	: African Animal Trypanosomiasis
BEF	: Bovine Ephemeral Fever
BCT	: Bovine Cerebral Theileriosis
BPAT	: Buffered Plate Agglutination Test
CBPP	: Contagious Bovine Pleuropneumonia
CCPP	: Contagious Caprine Pleuropneumonia
CFT	: Complement Fixation Test
DAAD	: German Academic Exchange Services
DAGs	: Directed Acyclic Graphs
ECF	: East Coast Fever
ELISA	: Enzyme Linked Immunosorbent Assay
FGD	: Focus Group Discussion
FMD	: Foot and Mouth Disease
FPA	: Fluorescence Polarisation Assay
GLMM	: Generalized Linear Mixed-effect Model
IACUC	: Institutional Animal Care and Use Committee
ICC	: Intra-cluster Correlation Coefficient
IREC	: Institutional Research Ethics Committee
IZSLER	: Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna
LPBE	: Liquid Phase Blocking ELISA
MAT	: Microscopic Agglutination ELISA
Mabs	: Monoclonal Antibodies
MCF	: Malignant Catarrhal Fever
MMNR	: Maasai Mara National Reserve
NSP	: Non-Structural Proteins
PCR	: Polymerase Chain Reaction
PPR	: Peste des Petits Ruminants
RBT	: Rose Bengal Plate
SEIR	: Susceptible-Exposed-Infected-Recovered
SPCE	: Solid Phase Competitive ELISA
SPBE	: Solid Phase Blocking ELISA
SPs	: Structural Proteins
SPSS	: Statistical Package for Social Sciences
SNPs	: Single Nucleotide Polymorphisms
VNT	: Virus Neutralization Test

ABSTRACT

Intact ecosystems can regulate the transmission of infectious diseases by maintaining the diversity of species in equilibrium. However, human driven land use change is a major driver of environmental change and can affect the emergence and the transmission dynamics of infectious diseases. This study investigated how land use change, and hence the extent of wildlife-livestock interactions affect the transmission dynamics of infectious diseases in the Maasai Mara ecosystem in Kenya, using foot and mouth disease virus (FMDV), *Brucella* spp. and *Leptospira* spp. as case study pathogens. Three ecological zones were selected along transect from the Maasai Mara National Reserve (MMNR) to settled areas with different land-use types and varying resulting levels of wildlife-livestock interactions. Areas surrounding the MMNR and wildlife conservancies represented zone 1 (areas with intensive wildlife-livestock interactions) while areas between 20-40 km away from the MMNR constituted zone 2 with moderate wildlife-livestock interactions. Zone 3 was represented by areas more than 40 km away from the MMNR where wildlife-livestock interactions are low. A participatory epidemiological study was first conducted to determine peoples' perceptions on prevalence, seasonality, and impacts on livestock production of infectious diseases of concern to pastoralists in the defined zones. For this objective, four villages were purposively selected in zone 1 and another two in zone 2. Data were collected in focus group discussions (FGDs) using participatory epidemiological methods and with each group having 8-13 participants. A cross-sectional study was also conducted to determine the seroprevalences of *Brucella* spp., *Leptospira* spp. and foot and mouth disease (FMD) among cattle herds raised in the area. Five villages were purposefully selected; two in zone 1, another two in zone 2 and one in zone 3. A total of 1,170 cattle sera were collected from 390 herds distributed across the zones and tested for antibodies against *Brucella abortus*, *Leptospira interrogans* serovar *hardjo* and non-structural 3ABC proteins (NSPs) of FMDV using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits. All sera samples were further tested for serotype-specific antibodies of FMDV using Solid Phase Competitive ELISA (SPCE) kits (IZSLER, Italy). The specific FMDV serotypes targeted included, A, O, South African Territory [SAT] 1 and SAT 2, known to be endemic in Kenya. Data on putative risk factors for transmission of the targeted pathogens were collected for each sampled herd using a household questionnaire. A compartmental baseline model with Susceptible-Exposed-Infected-Recovered (SEIR) epidemiological classes was also developed to simulate the theoretical transmission of FMDV between cattle and sheep hosts. Thereafter, a series of deterministic SEIR models were fitted using the baseline model framework to evaluate the effects of reactive and pre-emptive vaccination strategies in reducing the cumulative incidence of FMD. The results of the participatory study showed that groups associated wildlife presence with malignant catarrhal fever (MCF), FMD, East coast fever (ECF), African animal trypanosomiasis (AAT), and anthrax, but they did not identify such linkages with goat pox, bovine ephemeral fever (BEF), salmonellosis and bovine cerebral theileriosis (BCT). When data from all sites were combined for impact matrix scoring, MCF, anthrax, FMD, contagious bovine pleuropneumonia (CBPP), ECF and AAT, in decreasing order, were considered to cause the highest economic losses in livestock production. A Kruskal-Wallis test revealed a significant difference in FMD annual prevalence between cattle age groups ($p < 0.001$) and was the highest in animals > 4 years (median score of 32.5, range; 10-50). FMD had the highest impact on milk production, but in relation to its treatment costs, it was ranked second to CBPP. The overall apparent animal-level seroprevalences of *Leptospira* spp., *Brucella* spp.

and FMD were 23.5% (95% CI; 21.1-26.0), 36.9% (95% CI; 34.1-39.8) and 83.8% (95% CI; 81.5–86.2), respectively. Zones 1 and 2 (closer to the MMNR) had significantly higher seroprevalence of *Leptospira* spp. than zone 3 ($\chi^2 = 7.0$, $df = 2$, $p = 0.029$), while for *Brucella* spp., the seroprevalence was higher in zone 1 than in zones 2 and 3 ($\chi^2 = 25.1$, $df = 2$, $p < 0.001$). The seroprevalence of FMD was also higher in zone 1 than zones 2 and 3 ($\chi^2 = 116.1$, $df = 2$, $p < 0.001$). 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 ($\chi^2 = 0.90$, $df = 2$, $p = 0.639$). Both serotypes A and O were more prevalent in zones 1 and 2 than zone 3 (low interface area) while serotype SAT 1, was higher in zone 3 compared to other zones. The results of multivariable analyses identified animal sex (female) and zones (high interface area) as significant predictors ($p < 0.05$) of animal-level seropositivity of *Brucella* spp. while for *Leptospira* spp., important predictors of animal-level seropositivity were animal sex (female), zones (moderate interface area) and herds utilizing a communal grazing reserve. For FMD, animal sex (female), raising of cattle in zones with moderate and high wildlife-livestock interactions; mixing of cattle from multiple herds at watering points and pastoral husbandry practices were all identified as significant predictors of animal-level seropositivity. The results of the vaccination scenario analyses against FMD indicated that both cattle and sheep hosts should be vaccinated in the Maasai Mara ecosystem for vaccination campaigns to have the desired effect on this disease. Reactive vaccination of cattle alone, for example, with 100% coverage reduced the cumulative incidence of FMD by 4.23% and 0.04% in cattle and sheep populations, respectively, while when both host species were targeted, the cumulative incidence of FMD decreased by 4.43% and 2.15% in cattle and sheep hosts, respectively. The results also showed that reactive mass vaccination can substantially reduce the cumulative incidence of FMD if implemented immediately at the onset of the outbreak and with high coverage to compensate for the low vaccine efficacy. The cumulative incidence of FMD, for example, reduced by 3.18% and 1.61% in cattle and sheep populations, respectively, at day 1 of reactive mass vaccination with 75% coverage compared to 0.009% (cattle) and 0.005% (sheep) at day 15 with the same coverage. Overall, pre-emptive mass vaccination was found to be more effective in reducing FMD cumulative incidence than reactive vaccination even when the former was implemented at low coverage. At a low coverage of 25% in both cattle and sheep hosts, pre-emptive vaccination reduced the cumulative incidence of FMD by 75.5% and 10.3% in cattle and sheep populations respectively, when implemented 5 days before the outbreak. This study provides information on disease priorities that occur in the surveyed zones in the Mara ecosystem and which the locals must consider when accessing key ecosystem services such as water and pasture. The seroprevalences of *Brucella* spp., *Leptospira* spp. and FMD in cattle were higher in areas with moderate to high wildlife-livestock interactions than those with rare interactions. The observed differences in the seroprevalences of the targeted pathogens between zones can be considered while instituting routine disease control programs.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The Maasai Mara ecosystem located in Narok County in Kenya is part of the arid and semi-arid lands (ASALs) of Kenya's landmass that supports wildlife conservation and livestock production (Homewood *et al.*, 2012). Overall, livestock farming contributes to about 12% of Kenya's gross domestic product (GDP) (Kabubo-Mariara, 2009). The Mara ecosystem comprises the Maasai Mara National Reserve (MMNR) and the surrounding territories inhabited by diverse wildlife species that spill-out of the reserve. These territories are also inhabited by the Maasai pastoralists and their livestock herds comprising cattle, sheep and goats (Bedelian & Ogutu, 2017).

Over the past decade, the Maasai Mara ecosystem has undergone major land use changes due to increased human populations, settlement expansion, infrastructure development (e.g., roads, fencing) and land privatization (Ogutu *et al.*, 2016; Løvschal *et al.*, 2017). An example of these land use changes is the establishment of wildlife conservancies in areas adjacent to MMNR and increased crop-livestock mixed agriculture (livestock production and crop cultivation) in areas distant from the reserve (Nthiwa *et al.*, 2019). Whereas the establishment of wildlife conservancies provide a sustainable way of integrating wildlife conservation with livestock production (Løvschal *et al.*, 2019), the current type of land use is associated with many challenges including competition for ecological resources (e.g., pasture and water) between wildlife and livestock (Bedelian & Ogutu, 2017) and livestock depredation (Mukeka *et al.*, 2019). The close interactions between wildlife and

livestock in areas near wildlife conservancies or MMNR may also expose livestock to infectious pathogens that are transmitted or maintained by wildlife (Enström *et al.*, 2017; Nthiwa *et al.*, 2019). Foot and mouth disease (FMD), contagious bovine pleuropneumonia (CBPP), blue tongue, anthrax, trypanosomiasis, East coast fever (ECF), brucellosis and leptospirosis are examples of infectious livestock diseases putatively associated with wildlife in the area. Reverse transmission (spill back) of these infectious pathogens may also occur from livestock to wildlife if pasture and watering sources are shared between both. This is primarily due to environmental persistence of these pathogens (Aune *et al.*, 2012; Barragan *et al.*, 2017). Besides livestock, the human populations living in the area are also at risk of zoonotic exposure via spillover due to increased frequent interactions between wildlife and livestock (Alexander *et al.*, 2018). Human infections by zoonotic pathogens including brucellosis and leptospirosis mainly occur via close contacts with infected animals, but also through consumption of raw milk or dairy products contaminated with these pathogens (Adler & de la Peña Moctezuma, 2010; Seleem *et al.*, 2010). Targeted control of zoonotic pathogens in livestock could therefore mitigate transmission in humans because livestock are the main sources of infections in humans. This study investigated the transmission dynamics of FMD, *Brucella* spp. and *Leptospira* spp. in cattle in three zones of the Mara ecosystem with different land use types and varying levels of wildlife-livestock interactions to understand how different land use types and varying levels of wildlife-livestock interactions affect the exposure of livestock to these pathogens. The results can inform the development of sustainable control strategies in the area and contribute to the understanding of how pathogen-host diversity could potentially influence infectious disease risk and outbreaks.

1.2 Problem statement

While FMD, brucellosis and leptospirosis are important threats and constraints to livestock production in many pastoral areas (Seleem *et al.*, 2010; Knight-Jones & Rushton, 2013; de Vries *et al.*, 2014), there is limited work that has investigated how different land use types and varying levels of wildlife-livestock interactions affect the transmission dynamics of these pathogens among pastoral cattle herds raised in the Mara ecosystem. Knowledge on the epidemiology of these pathogens is also limited in livestock, wildlife and human populations in the area and indeed in many resource-poor areas due to lack of prioritization, lack of surveillance systems and poor diagnostic capacities (Smith *et al.*, 2014; Allan *et al.*, 2015; Ducrotoy *et al.*, 2017). Using FMDV, *Brucella* spp. and *Leptospira* spp. as case study pathogens, this study determined the transmission dynamics of these pathogens in cattle across three zones with different land use types and varying levels of wildlife-livestock interactions. Given the limited available data on leptospirosis and brucellosis in the area, and host-specific transmission parameters in wildlife, the study only developed a theoretical transmission model for FMD between cattle and sheep to evaluate the effect of animal vaccination in its transmission.

1.3 Justification and significance of the study

Livestock keeping is the main economic activity among the Maasai pastoralists. However, infectious diseases such as FMD, brucellosis and leptospirosis constraint livestock production and limit the locals from deriving maximum benefits from livestock and their associated products (Enström *et al.*, 2017; Nthiwa *et al.*, 2019). These diseases significantly affect the livelihood resilience of livestock-dependent households as they

cause both direct and indirect economic losses. FMD infections in cattle, for example, are associated with chronic heat intolerance, reduced animal's milk yields, reduced draught power, abortions and mortality, mostly in calves (Knight-Jones & Rushton, 2013). FMD outbreaks can also be devastating as they prevent farmers from accessing markets for their live animals or animal-source products (e.g., milk and meat) due to imposed trade embargos and quarantine that restricts animal movement (Knight-Jones *et al.*, 2017). Comparably, the burden of leptospirosis and brucellosis is unknown as surveillance and notification systems in the Mara ecosystem are lacking hence insufficient morbidity and mortality data. Both brucellosis and leptospirosis are also known to cause abortions, infertility, reduced animal's milk yields and weak calves following the first calving (Adler & de la Peña Moctezuma, 2010; Franc *et al.*, 2018). Information on the exposure levels of these pathogens within the targeted zones will underpin and guide the establishment of appropriate control strategies in the area. Ultimately, this will reduce food security vulnerabilities of the Maasai pastoralists through improved management strategies of these diseases.

1.4 Research questions

This study aimed to answer the following questions:

1. Does the level of knowledge, attitude and practices of the Maasai pastoralists on FMD, brucellosis and leptospirosis contribute to disease epidemiology in different zones of Mara ecosystem with varying levels of wildlife-livestock interactions?

2. Are seroprevalences and risk factors of FMD, *Brucella* spp. and *Leptospira* spp. in cattle the same in different zones of the Mara ecosystem with varying levels of wildlife-livestock interactions?
3. How effective is the vaccination of cattle alone versus vaccinating both cattle and small ruminants in the management of FMD in the Mara ecosystem?

1.5 Hypotheses

1. The level of knowledge, attitude and practices of the Maasai pastoralists on FMD, brucellosis and leptospirosis do not significantly contribute to disease epidemiology in different zones of the Mara ecosystem with varying levels of wildlife-livestock interactions.
2. The seroprevalences of FMD, *Brucella* spp. and *Leptospira* spp. in cattle do not differ significantly in different zones of the Mara ecosystem with varying levels of wildlife-livestock interactions.
3. The effectiveness in vaccination of cattle alone versus vaccinating both cattle and small ruminants in the management of FMD does not differ significantly in the Mara ecosystem.

1.6 Objectives

1.6.1 Overall objective

The overall objective of this study was to investigate how different land use types and varying levels of wildlife-livestock interactions affect the transmission dynamics of FMD, *Brucella* spp. and *Leptospira* spp. within the Mara ecosystem.

1.6.2 Specific objectives

The specific objectives of this study were:

1. To assess the level of knowledge, attitude and practices of the Maasai pastoralists on FMD, brucellosis and leptospirosis in different zones of the Mara ecosystem with varying levels of wildlife-livestock interactions.
2. To determine the seroprevalences and risk factors of FMD, *Brucella* spp. and *Leptospira* spp. in cattle in different zones of the Mara ecosystem with varying levels of wildlife-livestock interactions.
3. To develop a mathematical model and use the framework to assess the effectiveness of various vaccination strategies of FMD targeting cattle alone versus targeting both cattle and small ruminants in the Mara ecosystem.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Effects of land use changes on the transmission dynamics of infectious diseases

The increase of human population has prompted land use changes in many parts of the world in order to meet the demand for food, timber, fiber, shelter, water and other ecosystem services (Mastel *et al.*, 2018). Anthropogenic-driven land use change is a major cause of environmental change (Foley *et al.*, 2005; Patz *et al.*, 2008), and can also impact negatively on human and livestock health (Lambin *et al.*, 2010; Perry *et al.*, 2013). Land use change, for example, can lead to loss of wildlife species biodiversity due to ecological degradation and habitat loss or fragmentation (Ogutu *et al.*, 2009; Brearley *et al.*, 2013). The decline of wildlife species richness and abundance cause changes in ecosystem structure and can reduce the disease regulatory role (i.e., ‘dilution effect’) provided by intact ecosystems (Keesing *et al.*, 2010). A low diversity of wildlife species can also lead to increased infectious disease risks due to decline in wildlife hosts which may act as pathogen sinks without further transmission to definitive hosts or those that may prevent density-dependent transmissions rates by suppressing the population of the primary reservoir (Suzán *et al.*, 2009). For instance, the overfishing of a *Trematocranus placodon*, a cichlid that feeds on *Bulinus* gastropods snails led to the increase in population of the latter, and subsequent transmission of *Schistosoma* parasites among human populations living around lake Malawi (Stauffer *et al.*, 2006).

Examples of anthropogenic activities that influence or modify the transmission dynamics of infectious disease include, deforestation, irrigation, infrastructure development (e.g.,

dams and roads), agricultural activities (e.g., intensified livestock production), extraction of natural resources (e.g., mining, hunting, fishing and logging), habitat fragmentation or loss, urbanization and encroachment into forests and wildlife habitats (Murray & Daszak, 2013; Hassell *et al.*, 2017). Some of the pathways through which these land use activities influence infectious disease risks include causing changes in density and distribution of host populations, pathogens and vectors (Lambin *et al.*, 2010; McFarlane *et al.*, 2013). In Tana River County, for example, inter-epidemic patterns of Rift Valley fever (RVF) have been associated with irrigation which provides favorable conditions for breeding of *Aedes* spp., important vectors for RVF virus (Sang *et al.*, 2017; Mbotha *et al.*, 2018). Land use changes can also indirectly or directly influence the demography, behavior, movement and contacts between hosts, vectors and pathogens (Karesh *et al.*, 2012; Gottdenker *et al.*, 2014). For instance, land use practices such as protected areas, conservancies and crop-livestock agriculture can influence the level of interactions between species by either inhibiting or facilitating their movements and contacts (Caron *et al.*, 2013). This is because the patterns of ecological interactions between species (indirect or direct contacts) depend on biotic and environmental factors (Caron *et al.*, 2013). Therefore, different wildlife-livestock overlaps and interactions, ranging from physical separations by fences to unfenced areas where livestock and wildlife routinely interact, can influence the level of microbe transmission between host populations (Miguel *et al.*, 2017).

The emergence of infectious diseases attributed to different land use types may also be totally new in an area or to individuals of a specific host. It can occur either through cross-species transmission or extension of geographical coverage into modified or new habitats (Brearley *et al.*, 2013). It can also occur via pathogen spillover which involves initial

invasion and microbe establishment in new hosts (i.e., ‘host jump’) (Godfroid, 2018). This is illustrated by several viral pathogens such as the severe acute respiratory syndrome coronavirus (SARS-CoV-2), Ebola and middle East respiratory syndrome coronavirus (MERS-CoV) which infects humans and are thought to have originated from animals (Lai *et al.*, 2020). The targeted pathogens (*Brucella* spp., *Leptospira* spp. and FMDV) are other examples of pathogens maintained in wildlife reservoirs, but can spillover into livestock through close inter-species interactions (Miguel *et al.*, 2017; Plowright *et al.*, 2017).

2.2 Aetiology and diagnosis of brucellosis, leptospirosis and FMD

2.2.1 Brucellosis

Brucellosis is a neglected bacterial zoonotic disease that affects both livestock and humans (Ducrotoy *et al.*, 2017). The etiological agent of bovine brucellosis is a facultative intracellular gram-negative coccobacilli of the genus *Brucella* (Seleem *et al.*, 2010). Whereas *Brucella abortus* is the main etiological agent of brucellosis in cattle, *B. melitensis*, the species that mainly affects sheep and goats, may infect cattle if inter-species interactions between these species occurs (Seleem *et al.*, 2010). *Brucella* spp. preferentially localize and replicate within the reproductive tracts of both male and female animals. The high affinity for genital organs causes orchitis, epididymitis, abortions, weak offsprings, stillbirths and infertility (Letesson *et al.*, 2017). The disease also causes huge direct losses due to reduced animal’s milk yields, loss of draught power and poor weight gain (Franc *et al.*, 2018). The main clinical manifestation of brucellosis in humans include

fever, sweats, fatigue, headache, malaise, and joint aches, muscle and back pain (Dean *et al.*, 2012).

The conventional methods used in the *Brucella* infection diagnosis include; modified acid-fast staining, isolation of *Brucella* spp. pathogens through culturing and serological assays. Despite the high specificity of *Brucella* culture, the technique is slow, labour intensive and requires specialized growth media for the bacteria (Godfroid *et al.*, 2010). Most epidemiological surveys use serological assays such as Rose bengal plate test (RBT), fluorescence polarisation assay (FPA), serum agglutination test (SAT), buffered plate agglutination test (BPAT), complement fixation tests (CFT), enzyme immunoassays (e.g., indirect and competitive ELISA tests) and lateral flow immuno-chromatography tests (Godfroid *et al.*, 2010; Ducrotoy *et al.*, 2017). Among these serological assays, RBT, BPAT and ELISA tests are the most preferred assays in epidemiological investigations due to their simplicity and higher sensitivity (Godfroid *et al.*, 2010).

Polymerase chain reaction (PCR) based assays are also widely used to distinguish between various *Brucella* species (Yu & Nielsen, 2010). The PCR based tests include the use of single PCR primers to discriminate *Brucella* pathogens at the genus level. The genus-specific PCR methods target the *bcp31* gene encoding the 31kDA protein, outer membrane proteins (e.g., *omp2a*, *omp2b*, and *omp31*) or the 16S ribosomal ribonucleic acid (rRNA) gene sequences (Yu & Nielsen, 2010). Multiplex PCR assays using several oligonucleotide primers have also been developed for the discrimination of several *Brucella* species in a single test. Examples of these multiplex PCR assays, include the *abortus-melitensis-ovis-suis*, (AMOS) PCR (Bricker, 2002), and the Bruce ladder PCR

for the identification of nine pathogenic *Brucella* species including *B. abortus*, *B. melitensis*, *B. ovis*, *B. canis*, *B. suis*, *B. microti*, *B. inopinata*, *B. pinnipedialis* and *B. ceti* (Mayer-Scholl *et al.*, 2010).

2.2.2 Leptospirosis

Leptospirosis is an important bacterial disease that affects diverse hosts such as wildlife, humans and livestock (Allan *et al.*, 2015; Assenga *et al.*, 2015). It occurs in both urban and rural areas and is considered as one of the most widespread zoonoses globally, with incidences occurring either sporadically or in epidemics (de Vries *et al.*, 2014). Most of these outbreaks are associated with flooding following heavy rains (Lau *et al.*, 2010). The causative agent of bovine leptospirosis is a pathogenic spirochetes of the genus *Leptospira*, family leptospiraceae (de Vries *et al.*, 2014). There are more than 250 distinct pathogenic serovars (antigenic types) of *Leptospira* spp. identified through serological techniques and based on surface antigens (Evangelista & Coburn, 2010). These serovars are often grouped into more than 20 serogroups based on antigen relatedness (Bharti *et al.*, 2003). *Leptospira* spp. colonize and settle in the proximal renal tubules of host species. Infected animals become chronic carriers of these pathogens and are important sources of infections due to environmental contamination of soil or water sources through urinary shedding (Mwachui *et al.*, 2015). Both wildlife species and rodents are the main reservoirs of *Leptospira* spp., whereas cattle, dogs and pigs can act as temporary carriers for several months (Allan *et al.*, 2015). Cattle infections are mainly manifested by fever, reduced animal's milk yields, abortions, stillbirths, weak calves, infertility and renal failure (Adler & de la Peña Moctezuma, 2010; Lilenbaum & Martins, 2014).

The most common diagnostic methods of *Leptospira* infections include; bacterial culturing using urine or tissue samples, microscopic agglutination test (MAT), enzyme immunoassays and PCR based assays (de Vries *et al.*, 2014). The molecular-based assays aim at detecting *Leptospira* DNA using oligonucleotide probes targeting the 16S and 23S rRNA genes, universal targets for all *Leptospira* spp. (Faruque *et al.*, 2017). These probes include 5'-GACCCGAAGCCTGTCGAG-3' and 3'-GCCATGCTTAGTCCCGATT-AC5' that target 23S rRNA genes (De Roy *et al.*, 2014). The oligonucleotides 5'-GGCGGCGCGTCTTAAACATG-3' and 5'-CTTAACTGCTGCCTCCCGTA-3' (forward and reverse primers, respectively) target the 16S RNA genes (Matsui *et al.*, 2012). Recent studies have reported that the 16S rRNA is an important marker for the diagnosis of *Leptospira* spp. with specificity and sensitivity of 100% and 64%, respectively (Backstedt *et al.*, 2015). Nevertheless, the 23S PCR based assay has been found to be a better molecular marker than the 16S based PCR assay with 100% sensitivity (D'Andrea *et al.*, 2012). Although the use of these markers results in higher sensitivity and specificity than serological assays, the technique is relatively expensive for routine diagnostic procedures and lacks automated and standardized procedures for screening large sets of samples (Stoddard *et al.*, 2009).

2.2.3. Foot and mouth disease (FMD)

FMD is a highly infectious viral disease that affects numerous species of both cloven-hoofed livestock (e.g., cattle, sheep, goats, donkeys and pigs) and wild animals including the African buffalo, zebra and wildebeests (Wekesa *et al.*, 2015). In all species, FMD mainly manifests as acute febrile infirmity in the early phases of infection, lameness,

anorexia, salivation and the formation of lesions on the teats, feet and oral cavity (Arzt *et al.*, 2011). Whereas pathogenesis is also depended on the livestock species involved, pigs are more refractory to aerosol infections than cattle (Pacheco *et al.*, 2010).

The disease is caused by the foot and mouth disease virus (FMDV) of the genus *Aphthovirus*, within the family Picornaviridae (Bari *et al.*, 2014). The FMDV is icosahedral shaped and consists of a single-stranded positive-sense RNA with an approximately genome size of 8.5 kilo base pairs (kb) (Longjam *et al.*, 2011). The viral genome has three main functional groups, namely, the 5' noncoding regulatory region, the polypeptide coding region which has L, P1, P2 and P3 regions and the 3' noncoding region (Bari *et al.*, 2014). Translation of the viral genome results into a single polypeptide which upon post-translational modification, it is cleaved by viral proteases to form several non-structural proteins (NSPs) such as; L, ZA, ZB, ZC, 3A, 3B, 3C and 3D and four structural proteins (SPs) (i.e., VP1- 4) (Longjam *et al.*, 2011). The VP1-3 form the outer capsid while VP4 is located in the interior surface. Of the four SPs, VP1 is the main antigenic peptide of FMDV as it constitutes about 54% of the virus (Longjam *et al.*, 2011). In general, the FMDV exists in seven antigenically and genetically distinct serotypes (i.e., O, A, C, Asia 1 and the Southern African Territory [SAT] serotypes 1, 2 and 3) (Namatovu *et al.*, 2015). All FMDV serotypes produce indistinguishable clinical disease and recovery to one serotype does not offer immunity against other serotypes due to antigenic diversity (Grubman & Baxt, 2004; Bari *et al.*, 2014). Serotype O is more globally prevalent compared to other serotypes and has been responsible for major economically devastating epidemics in the United Kingdom, Korea and Japan (Brito *et al.*, 2017). Serotype C has a limited distribution but has also been reported in South America, North Africa, Europe

and Southern Asia (Sangula *et al.*, 2011). The serotypes SAT 1, 2 and 3 are mainly restricted to Africa while serotypes O and A have wider geographical distribution (Brito *et al.*, 2017). The major endemic serotypes in Eastern Africa include A, O, SAT 1 and SAT 2 (Bari *et al.*, 2014; Namatovu *et al.*, 2015).

2.2.3.1 Diagnosis of FMD using molecular-based tools

FMD spreads rapidly among susceptible animals and cause huge economic losses in the case of outbreaks. Therefore, timely and specific identification of the serotypes responsible for the outbreak is of great significance for effective control interventions (Knight-Jones *et al.*, 2017). FMD diagnostic tools include virus isolation through culturing, serological assays and molecular-based assays (Longjam *et al.*, 2011). Earlier molecular-based assays involved the hybridization of the FMD viral genome with a panel of radiolabeled DNA probes (Rossi *et al.*, 1988). The advent of PCR has led to the development of numerous automated reverse transcription PCR (RT-PCR) assays, that allow simultaneous discrimination of various FMD virus strains (Paixão *et al.*, 2008). These assays include the use of universal primers such as CP-U Biotin 5'-GATGCCCTTCAGGTACCCCGAGGTA-3', SP-forward 5'-CTTC-TCAGATCCCGA-GTGTC-3' and SP-reverse 5'-TGTTTCGTAGCGGAGCATG-3' targeting the conserved domains within the 5' untranslated region (UTR) and the RNA dependent RNA polymerase gene (3Dp^{ol}) for all the 7 serotypes (Collins *et al.*, 2002; Reid *et al.*, 2002). Results of these studies indicated differential sensitivity in the assays with the 3Dp^{ol} being more sensitive to the SAT serotype isolates while the 5' UTR detected more of serotype A (King *et al.*, 2006). Furthermore, the failure to detect some FMD isolates in both assays

was also observed due to the high mutation rate of the FMDV (Bari *et al.*, 2014). The inability of these assays to discriminate between various FMD viruses to serotype level has led to the development of multiplex PCR assays (mPCR) for differential diagnosis of FMD serotypes (Longjam *et al.*, 2011). Various studies have evaluated the diagnostic sensitivity of mPCR using tongue epithelia, cell culture and vesicular derived antigens and reported higher sensitivity in mPCR than conventional virus isolation (Giridharan *et al.*, 2005; Zinnah *et al.*, 2012). Multiplex PCR is limited by the generation of false positives due to carry-over of PCR products and thus not considered as an appropriate assay for routine screening of a large number of samples (Hoffmann *et al.*, 2009). The use RT-PCR to amplify the viral protein 1 (VP1) followed by the sequence analysis of the resultant PCR amplicons, is also widely used in FMD virus determination and phylogenetic analysis (Namatovu *et al.*, 2015; Wekesa *et al.*, 2015).

The development of DNA microarray technology also presents a unique capability to detect and characterize multiple microbes simultaneously in a single chip. This technology has a wider application in the evaluation of gene expression and also in the analysis of single nucleotide polymorphisms (SNPs) (Martín *et al.*, 2006). The DNA microarray method is an 'FMD DNA chip' based test consisting of several highly serotype-specific probes for concurrent detection and determination of all the seven FMD virus serotypes (Baxi *et al.*, 2006; Lung *et al.*, 2011). Besides high sensitivity and speed, the assay is robust as it uses multiple probes to identify each serotype and to readily cope with evolving new strains. It starts with RNA extraction from FMD virus infected materials, followed by PCR amplification of FMD virus serotypes using universal primers that amplify VP3-VP1-2A region of the viral genome. Thereafter, the PCR fragments

(radiolabeled target DNA) are hybridized to the oligonucleotide probes on the FMD DNA chips (Baxi *et al.*, 2006). The target-probe pairs are then visualized under ultraviolet illumination. The microarray assay has provided useful information on the molecular epidemiology of FMD virus. In particular, this has been achieved by the screening of point mutants within the G-H loop of VP1 protein, the genomic regions of the FMD virus that determine its antigenicity (Martín *et al.*, 2006).

2.2.3.2 Discriminating between infected from vaccinated animals by ELISA

Examples of serological tests used for routine screening of FMD antigen in tissues or for the detection of antibodies against the structural protein (SPs) and non-structural proteins (NSPs) of FMDV include Complement fixation test (CFT), virus neutralization tests (VNTs) and enzyme immuno-assays (Longjam *et al.*, 2011). Vaccination against FMD using multivalent FMD virus inactivated vaccines stimulates the production of antibodies against the structural proteins of FMDV (Bari *et al.*, 2014). Due to the absence of virus replication, antibodies against the NSPs are not elicited. Infected animals support FMD virus replication and thus the expression of NSPs. Upon recovery, such animals have antibodies to both SPs and NSPs. The presence of anti-NSPs (e.g., L, 2C, 3A, 3B, 3C and 3D) are reliable indicators of animal's previous exposure (i.e., infection-acquired immunity) or current infections (Longjam *et al.*, 2011). The 3ABC polypeptide is a highly immunogenic and is capable of discriminating between infected and vaccinated animals with high sensitivity and specificity (Biswal *et al.*, 2016).

2.2.3.3 FMDV Serotype determination by ELISA methods

The determination of FMDV serotypes responsible for immune response is based on detecting anti-SPs (VP1-4) which are expressed during vaccination or virus replication following infection (Cowled & Garner, 2008). The VP1 protein is the main antigenic site for the virus and is highly variable amongst different serotypes (Bari *et al.*, 2014). The serotype-specific assays recommended by the World Organization for Animal Health (OIE) include; liquid phase blocking Elisa (LPBE), solid phase blocking Elisa (SPBE), solid phase competitive Elisa (SPCE) and virus neutralization tests (VNTs) (Namatovu *et al.*, 2013). The sensitivity of these tests is high when the virus used is closely matched with the circulating field isolates. Previous studies have reported sensitivity scores in SPBE that are comparable to virus neutralization tests (VNTs) (Chénard *et al.*, 2003). Notably, both SPCE and LPBE could detect lower antibody titers than VNTs (Mackay *et al.*, 2001).

2.2.3.4 Pen-site FMD diagnostic approaches

Differential diagnosis of FMD infections under laboratory conditions can only be done in a specialized laboratory facility with well-trained personnel and requires stable reagents (Knight-Jones *et al.*, 2016). Laboratory-based methods also involve multistep handling or preparation of samples and stringent biosecurity measures during sample collection and transportation to minimize the spread of the FMDV (Rweyemamu *et al.*, 2008; Oem *et al.*, 2009). These drawbacks have led to development and validation of rapid, user-friendly and easy to perform pen-site diagnostic tests such as lateral flow devices and chromatographic strips based on monoclonal antibodies (MAbs) for detecting FMD virus

antigen (Jiang *et al.*, 2011). These assays allow the direct use of oral-pharyngeal vesicular fluids or epithelial suspensions with minimal sample processing. The pen-site diagnostic tools also reduce the amount of time needed for transport and laboratory screening of samples, thus allowing the prompt implementation of control interventions (Knight-Jones *et al.*, 2016).

CHAPTER THREE

3.0 KNOWLEDGE, ATTITUDES AND PRACTICES OF THE MAASAI PASTORALISTS ON MAJOR CATTLE DISEASES IN DIFFERENT ZONES OF MARA ECOSYSTEM WITH VARYING LEVELS OF WILDLIFE-LIVESTOCK INTERACTIONS

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3.1 Abstract

Livestock-wildlife interactions promote the transmission of a wide range of infectious diseases that constraint livestock production. Limited work has been done to determine peoples' perceptions on prevalence, seasonality, and impacts on livestock production of these diseases in areas with varying levels of livestock-wildlife interactions. This study used a participatory appraisal approach to find out and rank infectious diseases of concern to pastoralists in a zone of intense wildlife-livestock interaction and another zone with limited interactions. Four villages were selected purposively in areas with intensive cattle-wildlife interactions (zone 1), and another two in areas with low to moderate cattle-wildlife interactions (zone 2). Data were collected in focus group discussions (FGDs) using participatory appraisal approach; each group had 8-13 participants. Foot and mouth disease (FMD) was associated with increased mortality in zone 1 than zone 2 ($\chi^2 = 4.12$, $df = 1$, $p = 0.04$). The participants could also associate wildlife presence with malignant catarrhal fever (MCF), East coast fever (ECF), African animal trypanosomiasis (AAT), and anthrax, but they could not identify these linkages with goat pox, bovine ephemeral fever (BEF), salmonellosis and bovine cerebral theileriosis (BCT). Results of impact matrix scoring from all sites indicated that, MCF, anthrax, FMD, contagious bovine pleuropneumonia (CBPP), ECF and AAT, in that order, had the highest impact on livestock production. A Kruskal–Wallis test revealed a significant difference in FMD annual prevalence between cattle age groups ($p < 0.001$) and was highest in animals >4 years (median score of 32.5, range; 10-50). FMD had the highest impact on milk production, but based on veterinary costs (treatment costs), it was ranked second to CBPP. This study provides information on disease priorities that occur in the targeted zones in Mara ecosystem and which the local pastoralists must consider when accessing key ecosystem services such as water and pasture.

Keywords: Participatory techniques, cattle diseases, Maasai pastoralists, livelihoods.

3.2 Introduction

Livestock production is the main source of livelihoods and nutrition for over 300 million people residing in sub-Saharan Africa (Abdilatif *et al.*, 2018). In Kenya, it also contributes significantly to income, food and livelihood resilience of pastoral communities (Smith *et al.*, 2013). For many years, the Maasai pastoralists in Kenya have raised their livestock in wildlife inhabited areas where livestock-wildlife interactions occur mainly at watering and grazing areas (Bedelian & Ogutu, 2017). This has been the predominant production system for many years, but of late, there has been a shift towards the establishment of wildlife conservancies to support better utilization of the rangelands, both for livestock farming and wildlife conservation (Løvschal *et al.*, 2017). The revenues generated from wildlife-related tourism contribute a large proportion of the household income in these communities (Bedelian & Ogutu, 2017).

Establishment of wildlife conservancies has been recognized as a sustainable intervention for protecting wildlife and their ecosystems in areas with intense livestock-wildlife interactions. However, several challenges including the competition for resources and increased livestock-wildlife interactions, leading to increased transmission of infectious diseases, have been identified. Examples of animal health threats associated with wildlife in these locations include foot and mouth disease (FMD), blue tongue and zoonosis such as brucellosis and leptospirosis. Reverse transmissions of infectious diseases from livestock to wildlife have also been reported for various livestock diseases including bovine tuberculosis and brucellosis (Rhyan & Spraker, 2010). In such cases, wildlife may act as maintenance hosts or amplifiers of these pathogens with possible spillback to

livestock. Several studies have also demonstrated a substantial decline in wildlife populations due to increased competition for resources and degradation of ecosystems (Ogutu *et al.*, 2016). Such a decline of wildlife populations might also increase infectious disease transmission through indirect disease transmission processes. This is because a decline in animals that act as dead-end hosts for pathogens or those that limit contact between susceptible and infectious hosts erode the ‘dilution effect’ that is thought to limit disease emergence in stable ecosystems (Huang *et al.*, 2016).

This study aimed to determine the impact of livestock diseases on the livelihoods of the Maasai pastoralists in zones with varied degree of livestock-wildlife interactions. It specifically investigated whether the distribution of livestock diseases varied with the degree of livestock-wildlife interactions. These findings will help understand threats to animal health and livelihoods in the area and support prioritization of interventions to enhance cattle productivity and improve livelihoods and nutritional security for the Maasai pastoralists.

3.3 Materials and methods

3.3.1 Study area

This study was conducted within the Maasai Mara ecosystem in South Western Kenya (Figure 3.1). The area is a biodiversity hotspot for diverse wildlife species including the migrant zebra (*Equus quagga burchellii*) and blue wildebeests (*Connochaetes taurinus*) (Ogutu *et al.*, 2009). The Southern part of the ecosystem comprises the Maasai Mara National Reserve (MMNR), a protected area that joins the Serengeti National park in Northern Tanzania. The immediate areas surrounding MMNR have been converted into

wildlife conservancies, but are also utilized for livestock farming (Bedelian & Ogutu, 2017). These territories are also inhabited by the Maasai pastoralists.

The study area was stratified by land use type into two zones; the first (zone 1) bordered the Maasai Mara National Reserve (MMNR) and wildlife conservancies and therefore had intensive livestock-wildlife interactions, while the second (zone 2) was more than 20 km away from the MMNR and had low to moderate livestock-wildlife interactions, more intensive cattle production and some crop cultivation. The defined zones provided an ecological gradient that allowed analyses to be made on the risk of livestock diseases associated with land use change. People that lived in zone 1 utilized conservancies and MMNR for dry season grazing while those from zone 2 had largely adopted sedentary grazing lifestyles.

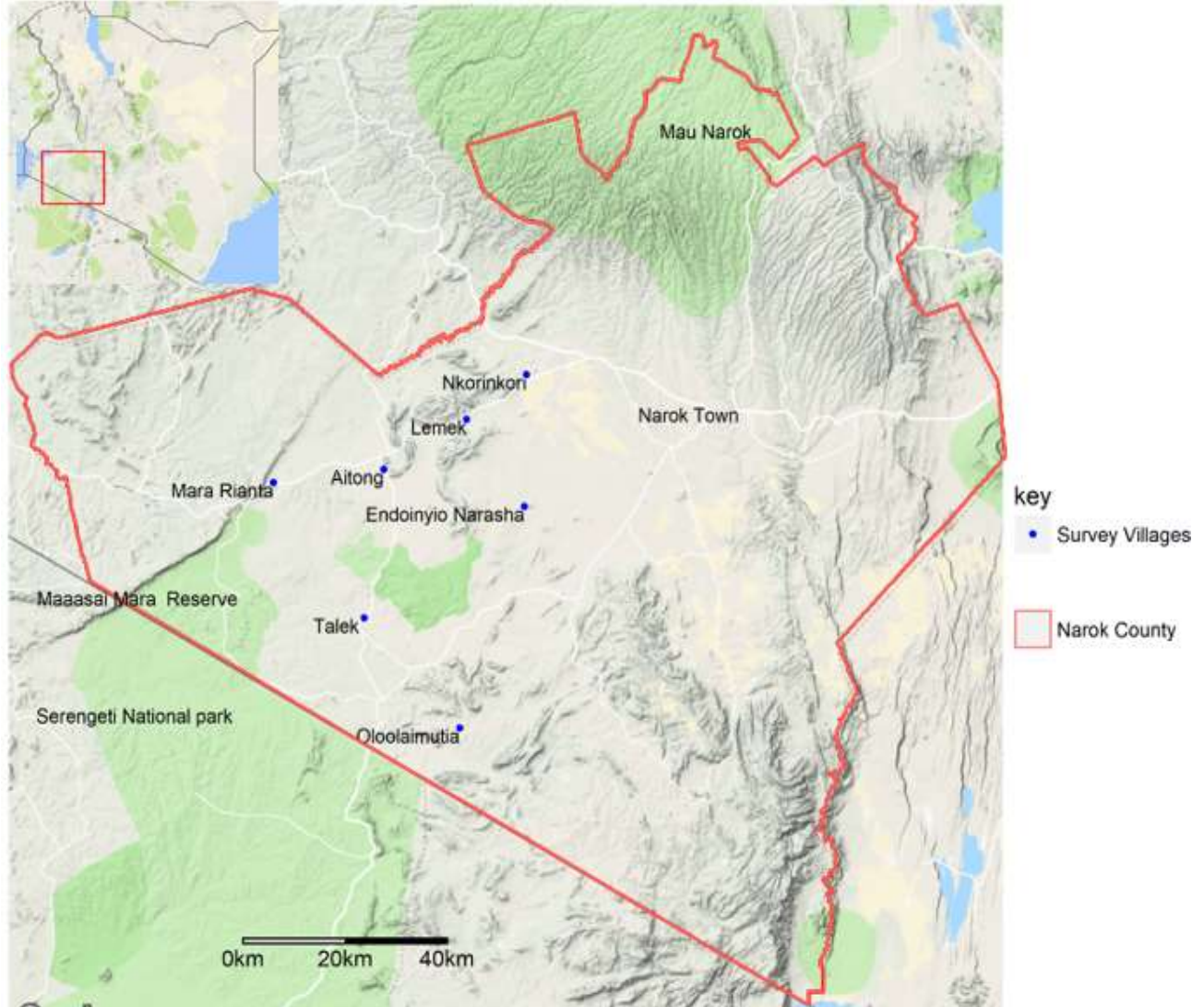


Figure 3.1: Map of Kenya showing the Maasai Mara ecosystem and the locations of the survey sites.

3.3.2 Compliance with ethical standards

Ethical and animal use approvals for this study were obtained from the International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (ILRI-IREC, reference number 2016-02, appendix 1) and the Institutional Animal Care and Use

Committee (ILRI-IACUC, reference number 2016–20, appendix 2), respectively. Additional approvals were also obtained from the veterinary services department and the local administrative authorities. All interviewed farmers provided verbal informed consent for cattle blood sampling and the questionnaire survey. The participants in the focus group discussions provided written informed consent for participating and for voice recording before discussions began.

3.3.3 Study design and selection of villages

The study used a cross sectional study design that involved four villages in zone 1 (i.e. Talek, Mara Rianta, Oololaimutia and Aitong [approximately 10 km away from the MMNR]) and two villages in zone 2 (i.e. Lemek and Endoinyio-Narasha villages) (Figure 3.1). These villages were selected purposefully to provide the required ecological gradient for the study.

3.3.4 Data collection

The study was conducted between September and October 2016. Focus group discussions (FGDs) were done in each target village. Preliminary visits to selected villages were planned with local authorities to schedule meetings with the farmers and introduce the project and its objectives. Purposive selection of participants was done to identify people who could provide reliable information on diseases and other ecosystem services in the area. Participants had to be 18 years old and above, residents in the village and either own livestock or come from households that kept livestock. FGDs were separated by gender in all the villages. In total, the number of FGDs conducted in zones 1 and 2 were 8 and 4 respectively, while based on gender, there were 6 FGDs for both men and women groups.

Each discussion group comprised 8-13 participants and discussions were conducted in the local Maasai language with the assistance of a translator. The discussions were guided by a checklist of open-ended questions that were pre-tested in three villages (not included in the main study) within the Mara ecosystem. The questionnaire checklist was refined before the start of the work. The participatory epidemiological tools used to collect data included semi-structured interviews, pair-wise ranking, disease impact matrix scoring, proportional piling and seasonal calendars (Catley *et al.*, 2012). During the FGDs, probing was used to ensure consistency of information obtained and to provide detailed information on items being discussed. Voice recorders were used to capture all discussions and to supplement notes taken at the time. Each group discussion lasted for at least 1.5 hours. Key questions addressed included livestock species kept and their respective benefits; livestock diseases and their impacts on livelihoods from livestock, with emphasis on FMD as an example of MMNR ecosystem disservice.

3.3.5 Herd species composition

Using proportional piling technique, FGD groups were asked to list the livestock species kept in the community and indicate the relative sizes of each species in an average herd. To do this, livestock species were listed on a flip chart and the group given 100 stones to distribute across species based on their relative abundance (i.e. the most abundant species received the highest number of stones). Stones allocated to each species were counted and participants were notified of the scores. Probing was used to discuss the numbers and to understand the reasons that informed the allocations made and for herd composition.

3.3.6 Livestock benefits and disease constraints to cattle production

Participants were asked to identify benefits they derived from each listed livestock species. Proportional piling was used with 100 stones with the same initial number of stones as described above (section 3.3.5), to determine the relative importance of each benefit to households' livelihoods. The number of stones allocated to each benefit was determined and discussions held to determine the reasons for the results of the exercise.

Participants were also asked to list the most common cattle diseases they had observed in their herds in the previous year (i.e. between September 2015 and October 2016). The identification of these diseases was based on recall and recognition memory as the farmers do not keep animal medical records. In general, pastoralists have rich indigenous veterinary knowledge that is based on shared information, oral tradition and long-life experiences with livestock keeping (Catley *et al.*, 2012). Using the list generated, pair-wise ranking was used (comparing two diseases at a time) to identify the one that was more common in livestock in the reference period. To help complete this exercise, a simple matrix that had disease names on the X and Y axes was designed. A disease that was perceived to be prevalent received the highest scores. Probing was used to identify reasons that underlined the observations made.

Seasonal calendars were also developed and used to define seasonal occurrence patterns of the prioritized diseases (Catley *et al.*, 2012). Further, discussions were held to identify potential risk factors for each disease and the role of wildlife in the transmission of listed diseases. In this session, participants were also asked to identify other constraints that affected livestock production in each village.

3.3.7 FMD annual age-specific prevalence and mortality

FMD was used as a case study disease to determine perceptions of how cattle-wildlife interactions affect animal disease prevalence. Proportional piling was used to estimate annual age-specific prevalence and mortalities due to FMD. Groups were asked to categorize cattle in a herd into various age groups using Maasai local names. The identified age groups were written on a flip chart and the group provided with 100 stones (representing herd size) to allocate them to age group based on their relative sizes. Using the scores allocated to each age group, participants were asked to further divide the stones into two piles representing the proportion of animals that remained healthy in the past year versus those that got FMD. Each pile of stones corresponding to FMD-infected cattle was further subdivided to show the proportion that survived the infection and those that died (case fatality). This exercise was repeated for all age groups and probing done to determine reasons that supported the scores allocated to each pile.

The market value of cattle with and without FMD was also estimated. Each group was asked to give the prices of cattle when healthy and when infected for each age group. The prices obtained were based on group consensus.

3.3.8 Impacts of livestock diseases on livelihoods from livestock

The impact that each of the identified disease had on the livestock-associated benefits was determined using disease impact matrix scoring. A matrix comprising prioritized diseases on the X-axis and livestock benefits on the Y-axis was developed. The exercise started with the ranking of the benefits by importance along the Y-axis; a benefit that was highly ranked received more scores than those that were not. The next step involved distributing

the scores already apportioned to each benefit across the diseases along the X-axis; a disease that had the highest impact on a given benefit received higher scores than those that did not. The overall disease impact scores were derived by adding up all the scores that each disease got. This approach allowed the generation of weighted scores as diseases that had the highest impact on a benefit that was highly preferred was identified as being important in the area.

Finally, the relative costs of treating FMD compared to the other listed diseases were indicated using 100 stones. Scores of 0 and 100 represented very low and very high veterinary costs, respectively.

3.3.9 Data management and analysis

Semi-quantitative data obtained from scoring and ranking exercises were entered into a database designed using MS Excel (Microsoft® Excel, Washington, 2013) and exported into Statistical Package for Social Sciences (SPSS), version 22.0 (Corp, 2013) for analysis using non-parametric and descriptive statistical methods. The analysis involved computing percentages, medians and ranges of the scores. Kendall's coefficient of concordance (W) was used to assess the level of agreement between groups as follows; weak agreement, $W < 0.26$, $P > 0.05$; moderate agreement, $W = 0.26-0.38$, $P < 0.05$; strong agreement, $W > 0.38$, $P < 0.01$ (Ayele *et al.*, 2016). Kruskal-Wallis test was used to compare the median scores between zones, gender and diseases.

3.4 Results

3.4.1 Livestock species

Sheep, cattle, goats, chicken and donkeys were identified by all the groups as the common livestock species kept in the target zones (Table 3.1). Their relative proportions determined from proportional piling exercises did not differ by gender of participants or zone as indicated by the strong overall degree of agreement between groups (Kendall's $W = 0.99$, $P = < 0.001$, $n = 12$ FGDs). The proportions of sheep and cattle were perceived to be higher in both zones compared to those of the other livestock species. Reasons given for the higher preference for sheep included better drought tolerance, steady production of milk even when cattle had been moved to dry season grazing areas in search of pastures, could be slaughtered at home, reproduced frequently - at least twice annually, and could be given out as gifts. Cattle, on the other hand, provided more income compared to sheep and were sold for capital expenses.

The relative proportions of goats, donkeys and chicken were lower than those of sheep and cattle. Goats were perceived to be more susceptible to diseases such as “*olodua*” (used for both enterotoxemia and peste des petits ruminants [PPR]), “*olomorooj*” (goat pox) and “*orkipei*” (contagious caprine pleuropneumonia [CCPP]). Donkeys were used for draught power to transport water and firewood and were more likely to be stolen than the other livestock species. Predation and diseases were identified as the main challenges in poultry production.

Table 3.1: Median scores and their respective ranges obtained from ranking livestock species kept by the Maasai in the Mara ecosystem, Kenya.

Zones	Livestock species				
	Cattle	Sheep	Goats	Chicken	Donkey
Zone 2 ^a	27.5 (25, 33)	38 (35, 44)	20 (15, 20)	9 (7, 12)	5 (4, 6)
Zone 1 ^b	27.5 (21, 32)	41 (34, 45)	18.5 (16, 20)	10 (8, 20)	5 (3, 8)
Overall scores (n = 12)	27.5 (21, 33)	39.5 (34, 45)	19 (15, 20)	9.5 (7,20)	5 (3, 8)

^aArea with low to moderate cattle-wildlife interactions.

^bArea with intense cattle-wildlife interactions.

n, Number of FGDs (12) that participated in the proportional piling.

3.4.2 Ranking of benefits from livestock

Table 3.2 gives overall median scores on perceived benefits from livestock by zone. In descending order of importance, the study identified, income from the sale of livestock, milk, employment, payment of bride price, meat and social status associated with livestock ownership as the most important livestock livelihoods. It was common for the local producers to buy young or emaciated animals and fatten them for sale. Other benefits such as hides for clothing and the use of livestock for draught power were regarded as being least important. A Kruskal–Wallis test comparison of the median scores for the livelihoods from livestock, showed no statistically significant differences by zone ($p > 0.05$), apart from meat consumption ($p = 0.006$) which was apparently higher in zone 2

than zone 1. All the groups had a high level of concordance on the generated median scores for the benefits ($W = 0.78$; range; 0.54 –1.0, $n = 12$ FGDs) and there were no gender differences observed.

Table 3.2: Overall relative importance of livestock benefits, results obtained by proportional piling

Zones		Benefits								
		Milk consumption	Meat consumption	Income	Bride price	Social status	Investment	Employment	Draught power	Hides
Zone 2 ^a	Md (Mn-Mx)	23 (17-30)	11 (10-16)	33 (32-50)	10.5 (7-20)	6 (4-14)	_d_	21 (0-21)	3 (1-6)	3 (3-3)
	N	4	4	4	4	3		1	4	2
Zone 1 ^b	^c Md (Mn-Mx)	20 (6-32)	6.5 (2-9)	34 (23-68)	14 (5-18)	7 (1-12)	17 (0-17)	12 (4-21)	4 (1-9)	3 (2-3)
	N	8	8	8	7	8	1	6	6	4
Total	Md (Mn-Mx)	21 (6-32)	8 (2-16)	33 (23-68)	11 (5-20)	7 (1-14)	17 (0-17)	12 (4-21)	3 (1-9)	3 (2-3)
	N	12	12	12	11	11	1	7	10	6

^aArea with low to moderate cattle-wildlife interactions.

^bArea with intense cattle-wildlife interactions.

^c Md, median; Mn, minimum; Mx, maximum.

^d Dash (-) means that the benefit was not mentioned hence not included in proportional piling.

n, Number of FGDs that contributed data to that benefit. Median scores for investment and employment as a benefit was not compared between zones and gender due to few cases.

3.4.3 Prioritization of livestock diseases

The participants identified MCF, ECF, FMD, CBPP and AAT as the five most prevalent diseases that affected cattle in the area in the previous year. Bovine ephemeral fever (BEF), anthrax, pox, salmonellosis (“*orsetet*”) and diseases with nervous syndromes such as bovine cerebral theileriosis (BCT), locally called “*ormilo*”, were least prevalent. No differences were noted on the spectra of diseases reported between zones and by gender. On seasonal occurrence patterns, FMD, CBPP, AAT, anthrax and salmonellosis had high incidences during dry than the wet season, while ECF, BEF and BCT were observed in wet than dry season. The perceived seasonality of these diseases did not differ between gender and zone. Most groups associated the transmission of FMD, ECF, AAT, CBPP and anthrax with the African buffalo (*Syncerus caffer*), blue wildebeests (*Connochaetes taurinus*) and elephants (*Loxodonta Africana*). MCF transmission was primarily associated with wildebeests’ calving period, occurring mainly between the months of November and March. The groups did not link the transmission of BEF, BCT, pox and salmonellosis with wildlife. They identified wet season and tall grasses as risk factors for BCT, dirty water and grass for salmonellosis and high densities of mosquitoes and muddy cattle sheds for BEF.

Other than diseases, the groups reported recurrent droughts, lack of water, inadequate veterinary services, wildlife-human conflicts such as predation and competition for resources including water and grass, restriction of animal movements by fencing, lack of enough markets and livestock theft as other constraints to livestock production in general.

3.4.4 FMD prevalence, mortality and impacts on market value

The groups identified three main cattle age groups namely; calves (“*elasho*” <1 year), weaners (“*olaram*” 2-3 years) and mature adults (“*nkishu sapukin*” >4 years). The overall median proportion of cattle in the various age groups over the last 1 year showed that, those above 4 years constituted the largest percentage in the herd structure with 50% (range; 45-70). Calves and weaners were 20% (10-30) and 30% (20-30), respectively. The annual median prevalence of FMD was the highest amongst cattle >4 years with 32.5% (range; 10-50), against 18.5% (10-25) and 12.5% (7-25) in weaners and calves respectively. A Kruskal–Wallis test comparison of these median scores indicated a significant difference in FMD annual prevalence between the cattle age groups ($p < 0.001$), but not between weaners and calves.

Slightly higher mortalities associated with FMD were observed amongst calves with median scores of 4.5% (range; 2-15) compared to weaners (0.5%; 0-10) and mature adults (1%; 0-15). The annual age-specific median prevalence and mortality estimates for FMD did not differ significantly by gender. All the 12 FGDs had a strong level of agreement ($W = 0.76$, $p < 0.001$) for estimates on herd structure and annual age-specific FMD prevalence. The median scores for FMD prevalence and mortality estimates between zones, were only significant for mortality estimates (Kruskal-Wallis test, $p = 0.041$). The median scores for FMD associated mortalities in cattle were respectively, 3.0% (range; 0-15) and 0.0% (range; 0-5) in zones 1 and 2, while the annual prevalence estimates were 18% (range; 7-50) and 20% (range; 10-50) respectively.

The median value of healthy cattle (without FMD) was US\$ 300 (range; 250-700) for mature adults compared to weaners (US\$ 200; 100-300) and calves (US\$ 100; 50-175). The reduction in the market sale value of FMD-infected cattle was estimated to be the highest in mature adults (median losses of US\$ 175, range; 75-370) while the value of calves and weaners would decrease by US\$ 45 (range; 20-100) and US\$ 60 (range; 20-150) of its normal value respectively. There were no significant differences between zones and gender on the given prices of cattle when healthy and when FMD infected. The overall agreement between groups for the given prices was high ($W = 0.92$, $p < 0.001$, $n = 12$ FGDs).

3.4.5 Disease impact matrix scoring results

Disease impact matrix scoring technique was used to rank cattle diseases based on their impacts on the livestock benefits. Reported impacts included reduction on milk production, decline in income from the sale of animals and increased veterinary costs (Table 3.3). All the diseases identified were perceived to reduce milk production. These diseases were ranked in a descending order based on their impacts on milk production as FMD, CBPP, AAT and MCF. This ranking was consistent between groups ($W = 0.49$, $p < 0.001$, $n = 12$); no significant differences were therefore observed on these impact scores by zone and gender. The impact of AAT was associated with frequent infections, while MCF was associated with high mortalities in livestock including those that were lactating. MCF, CBPP, FMD, anthrax and goat pox, in decreasing order, were perceived to reduce income from the sale of live animals. Participants indicated that MCF, pox and anthrax were difficult to control, and therefore caused extensive case fatalities, because of

unavailability of drugs and vaccines. Both FMD and CBPP reduced income and milk as they infected many cattle within herds with high case fatality.

Table 3.3: Overall summarized disease impact matrix scoring results

Disease Impact Scoring	FMD Md (Mn- Mx) ^a	MCF Md (Mn- Mx)	Anthrax Md (Mn- Mx)	ECF Md (Mn- Mx)	CBPP Md (Mn- Mx)	Pox Md (Mn- Mx)	AAT Md (Mn- Mx)	BEF Md (Mn- Mx)	Salmonellosis Md (Mn-Mx)	BCT Md (Mn- Mx)	Total Md Md (Mn- Mx)
Reduced milk (W=0.16)***	4.5 (1-11)	2.5 (0-6)	0 (0-4)	0 (0-3)	4 (0-20)	0 (0-2)	3.5 (0-9)	0 (0-3)	0 (0-4)	0 (0-0)	1 (0-20)
Meat reduction (W=0.60)***	0 (0-4)	0 (0,0)	3.5 (1-11)	0 (0-2)	2.5 (0-5)	0 (1-3)	0 (0-3)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-11)
Reduced income (W=0.01)*	6.5 (0-18)	10 (0-25)	4 (0-15)	2 (0-16)	7.5 (2-50)	3.5 (0-9)	1 (0-8)	0 (0-5)	2 (0-2)	0(0-0)	3 (0-50)
Reduced bride price (W=0.27)***	2 (0-9)	3.5 (0-6)	2 (0-5)	0 (0-6)	2 (0-5)	1 (0-3)	0 (0-2)	0 (0-1)	0 (0-1)	0 (0-0)	0 (0-9)
Reduced social status (W=0.54)***	0 (0-12)	4 (0-6)	0 (0-3)	0 (0-3)	2 (0-6)	0 (0-2)	0 (0-2)	0 (0-0)	0 (0-1)	0 (0-0)	0 (0-12)
Reduced draught power (W=0.89)***	0 (0-5)	0 (0,0)	0 (0-5)	0 (0-0)	0 (0-0)	0 (0-0)	3 (0-6)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-6)
Reduced employment (W=0.29)***	2 (0-7)	4 (0-13)	0 (0-3)	0 (0-2)	3 (0-6)	0 (0-3)	0 (0-0)	0 (0-0)	1 (0-9)	_ ^b	0 (0-13)
Reduced hides for clothing (W=0.94)***	0 (0)	0 (0,0)	2.5 (0-3)	0 (0-0)	0 (0-0)	2 (1-3)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-3)
Reduced investment	1 (1,1)	8 (8,8)	4 (4,4)	0 (0-0)	4 (4,4)	_ ^b	0 (0-0)	_ ^b	0 (0-0)	_ ^b	1 (0-8)
Veterinary costs (W=0.07)**	19 (0-30)	0 (0-16)	6.5 (0-20)	11 (0-46)	33 (9-78)	0.5 (0-10)	10 (0-48)	2 (0-10)	3 (0-10)	0 (0-0)	9 (0-78)

FMD, foot and mouth disease; MCF, malignant catarrhal fever; ECF, east coast fever; CBPP, contagious bovine pleuropneumonia; AAT, African animal trypanosomiasis; BEF, bovine ephemeral fever; BCT, bovine cerebral theileriosis.

^a Md, median scores; Mn, minimum; Mx, maximum values are shown in parentheses.

^b The benefit was not mentioned hence disease impact on the benefit was not scored.

W = Kendall's coefficient of concordance in 12 FGDs (*p > 0.05; **p < 0.05; ***p < 0.001). Weak agreement, W < 0.26, P > 0.05; moderate agreement, W = 0.26-0.38, P < 0.05; strong agreement, W > 0.38, P < 0.01. W value for investment was not calculated due to few cases. The benefit was also not compared by gender and zones.

3.5 Discussion

This study used participatory epidemiological (PE) tools to prioritize diseases that affect cattle herds in Mara ecosystem. PE tools have been widely used by researchers to investigate animal health-related topics in resource-poor areas (Kimaro *et al.*, 2017; Abdilatif *et al.*, 2018). The results of this study showed that livestock production contributes significantly to the livelihoods of the Maasai, consistent with other findings (Bellet *et al.*, 2012; Ayele *et al.*, 2016). The main livestock derived benefits identified by participants are aligned with findings reported in previous studies (Jibat *et al.*, 2013). Sheep and cattle were prioritized as the most important domestic species that sustained households' livelihood in the area. These species were kept in large stocks as a strategic tool to sustain milk and meat production, which constitute an important diet for pastoralists (Smith *et al.*, 2013). However, with increasingly recurrent droughts in the area, sheep were currently a more preferred livestock species as they performed better than cattle in drought situations and where feeds are scarce.

MCF, ECF, FMD, CBPP and AAT were prioritized as the most frequently occurring diseases in cattle herds in the past year. These results affirm previous findings in the region (Kairu-Wanyoike *et al.*, 2014; Kimaro *et al.*, 2017). Important zoonotic diseases such as brucellosis were not identified despite recent reports of high seroprevalence in cattle within the high interface zone (Enström *et al.*, 2017). The fact that this disease was not mentioned by participants could be explained by the lack of awareness or lack of distinct clinical manifestations that allow differential diagnosis with other diseases.

The perception that all livestock diseases mentioned were prevalent all year round suggests endemic stability, and could be due to the absence of comprehensive

vaccination strategies, limited veterinary services, high costs of vaccines and drugs, and limited surveillance systems in the Maasai Mara ecosystem. The high incidence of FMD and CBPP reported in cattle during dry seasons could be due to the congregation of livestock at watering and grazing resources during these periods, which may increase the risks of contact, infection and spread (Alhaji & Babalobi, 2016; Miguel *et al.*, 2017). The results on the high incidence of anthrax in dry or drought seasons agree with other studies in Tanzania (Mwakapeje *et al.*, 2018) and Uganda (Wafula *et al.*, 2008). This could be due to overgrazing in dry season leading to increased ingestion of anthrax spores. Overgrazing could also open land for excavation of spores from the soil and their possible dispersal. Due to the robust survival of anthrax spores in the soil, environmental contamination remains an important risk factor for transmission (Turner *et al.*, 2013). Furthermore, dry season exposes animals to environmental stressors (e.g., heat stress) and seasonal nutritional stress which can decrease the ability of animals to mount effective immune responses following exposure, thereby increasing their susceptibility to low anthrax infective dosages (Turner *et al.*, 2013; Mwakapeje *et al.*, 2018). The transmission dynamics of ECF, BEF and BCT are complex due to the involvement of arthropod vectors. The major vectors are *Rhipicephalus appendiculatus* for ECF and BCT; and mosquitoes, *Aedes* spp. and *Culex* spp. and midges, *Culicoides* spp. for BEF. The seasonal patterns reported by groups for these diseases reflect the expected seasonal changes in vector densities which often rise just after the rainy season (Walker & Klement, 2015; Bett *et al.*, 2017). The observed seasonality of AAT agrees with Kimaro *et al.* (2017), and may be associated with grazing of cattle in the MMNR, conservancies and bushy areas (favorable ecological conditions for tsetse flies), thus increasing contacts between vectors, cattle and wildlife reservoirs of trypanosomes.

Perceptions on the potential role of wildlife in the epidemiology of MCF, FMD, ECF, AAT, and anthrax is consistent with modern veterinary literature (Kock *et al.*, 2014; Miguel *et al.*, 2017). Furthermore, the information provided was relatively homogenous between zones. The African buffalo, (*Syncerus caffer*), for example, was listed by all groups as an important reservoir of diseases shared with cattle. This finding supports earlier reports that have demonstrated the transmission of SAT (South African Territory serotypes 1, 2 and 3) FMDV serotypes to cattle sharing water and grazing resources with buffalo (Miguel *et al.*, 2017). As other wildlife species cannot maintain FMD viruses for long periods of time, they can act as transient sources of infections when actively infected (Vosloo & Thomson, 2017a). Although wildlife species; elephants and African buffalo were identified as reservoirs of CBPP, this information is not supported by the current scientific evidence. CBPP is primarily a disease of cattle and water buffalo (*Bubalus bubalis*) and the role of the listed wildlife species in its epidemiology is not fully clarified (Smith *et al.*, 2017). This existing ethno-knowledge provide important information that could form the basis for further investigation. According to participants, wildlife does not play a role in the epidemiology of BEF, pox, and BCT. This indicates a knowledge gap given that pathogens responsible for these diseases have been confirmed in wildlife (Mentaberre *et al.*, 2013; Walker & Klement, 2015).

The priority diseases were relatively homogenous and strongly agreed between groups of the targeted zones. The lack of significant differences in the perceived disease risk between zones could be due to limited spatial and temporal resolution considered in this study, which might have been inadequate to show any difference or may be, PE tools are not sensitive for determining small changes in risk between contiguous areas

(Catley *et al.*, 2012). It is also possible that the unrestricted animal movement between zones (Bedelian & Ogutu, 2017), disseminated diseases across the area.

The higher prevalence of FMD in cattle >4 years than other age groups, may be explained by the different grazing strategies used for cattle in various age groups. Weaners and adult cattle >4 years were more likely to be exposed to FMD virus as they frequently contacted other herds and wildlife during grazing, while for calves, the low prevalence could be due to protective effects of maternally inherited antibodies that wanes off as age increases (Elnekave *et al.*, 2016). The median age-specific mortalities for FMD were reported highest in calves and decreased with increasing cattle age. The higher mortality in calves than other cattle age groups could be due to FMD induced myocarditis (Sobhy *et al.*, 2018).

Based on matrix scoring, MCF, FMD, CBPP, anthrax, ECF and AAT had the greatest impact on cattle benefits, mostly on milk production and income from livestock sales. These impacts could have caused huge burden to the households' dependent on livestock for milk production and income generation on daily basis.

3.6 Conclusion

In general, the dynamic interactions between livestock, wildlife and environment in the livestock-wildlife interfaces promote transmission of multiple infectious diseases. Some of these diseases, particularly those transmitted through direct contact are more prevalent during the dry season while those transmitted by vectors are more prevalent just after the wet season. From an ecological perspective, infectious diseases are considered as an ecosystem disservice which pastoralists must consider or trade-off

with other benefits such as pasture and water. This study provides information on disease priorities that affect pastoral herds in the defined ecologies, especially where livestock and wildlife interact. Analyses on ecosystem services and trade-offs particularly in pastoral areas should attempt to quantify the impacts of multiple diseases that occur in defined localities to obtain more accurate findings. In addition, determining the prevalence of co-infections would also guide the development of more effective interventions including building community's capacity on disease surveillance for sustained control. Veterinary interventions such as vaccination could also be deployed for multiple diseases. Vaccinations for FMD, could for instance, be conducted together with those of CBPP and ECF provided there are no interferences between vaccines being used. This would drastically reduce the unit cost of deployment of each dose of vaccine. Finally, the differences observed between zones on the prevalence of diseases could be considered while instituting routine disease control programs. Vaccination campaigns could for instance, be intensified in zone 1 (with high livestock-wildlife interactions) than in the other areas e.g. zone 2.

CHAPTER FOUR

4.0 SEROPREVALENCE AND RISK FACTORS OF *LEPTOSPIRA* SPP. AND *BRUCELLA* SPP. IN CATTLE AT THE WILDLIFE-LIVESTOCK INTERFACE AREA IN MAASAI MARA, KENYA

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4.1 Abstract

A cross-sectional study was conducted to determine the seroprevalence of *Brucella* spp. and *Leptospira* spp. and risk factors of exposure in cattle in three zones with varying land use types and wildlife-livestock interactions. Five villages were selected purposively; two in areas with intensive livestock-wildlife interactions (zone 1), another two in areas with moderate livestock-wildlife interactions (zone 2) and one in areas where wildlife-livestock interactions are rarer (zone 3). Sera samples were collected from 1,170 cattle belonging to 390 herds in all the zones and tested for antibodies against *Brucella abortus* and *Leptospira interrogans* serovar *hardjo* using ELISA kits. Data on putative risk factors for seropositivity of these pathogens in cattle was collected using a questionnaire. The overall apparent animal-level seroprevalence of brucellosis and leptospirosis were respectively, 36.9% (95% CI; 34.1-39.8) and 23.5% (95% CI; 21.1-26.0). *Brucella* spp. seroprevalence was higher in zone 1 than in zones 2 and 3 ($\chi^2 = 25.1$, $df = 2$, $p < 0.001$). Zones 1 and 2 had significantly higher *Leptospira* spp. seroprevalence than zone 3 ($\chi^2 = 7.0$, $df = 2$, $p = 0.029$). Results of multivariable analyses identified animal sex (female) and zones (high interface area) as significant predictors ($p < 0.05$) of animal-level seropositivity of *Brucella* spp. For *Leptospira* spp., important predictors of animal-level seropositivity were animal sex (female), zones (moderate interface area) and herds utilizing a communal grazing reserve. The seroprevalences of *Brucella* spp. and *Leptospira* spp. in cattle were higher in areas with high wildlife-livestock interactions than those with rare interactions.

Keywords: *Brucella* spp., *Leptospira* spp., Seroprevalence, Land use change, Wildlife-livestock interface

4.2 Introduction

Brucellosis and leptospirosis are neglected bacterial zoonotic diseases of veterinary and public health importance worldwide (Seleem *et al.*, 2010; de Vries *et al.*, 2014). In livestock-dependent households, these diseases cause direct economic losses due to the reduction in animal's milk yields, abortion and infertility (Adler & de la Peña Moctezuma, 2010; Franc *et al.*, 2018), significantly affecting the well-being of communities whose livelihood depend on livestock. Bovine brucellosis is caused by facultative intracellular Gram-negative coccobacilli of the genus *Brucella* (Seleem *et al.*, 2010). Whereas *Brucella abortus* is the main causative agent of bovine brucellosis, *Brucella melitensis*, the species that primarily affects sheep and goats, can occasionally infect cattle (Seleem *et al.*, 2010). Bovine leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira* (de Vries *et al.*, 2014).

Knowledge on the epidemiology of these pathogens is limited in livestock, wildlife and human populations in the Maasai Mara ecosystem (in Kenya) and indeed in many resource-poor areas due to lack of prioritization, poor surveillance systems and diagnostic capacities (Allan *et al.*, 2015; Ducrotoy *et al.*, 2017). The Maasai Mara ecosystem has a rich biodiversity of wildlife and a thriving tourism industry that provides additional livelihoods to the local people (Bedelian & Ogutu, 2017). In recent years, the area has undergone major land use changes due to increased human populations, infrastructure development (e.g., roads, fencing) and land privatization (Ogutu *et al.*, 2009; Løvschal *et al.*, 2019). An example of these changes is the establishment of wildlife conservancies in areas adjacent to Mara reserve and increased mixed farming (livestock production and crop cultivation) in areas further away from the reserve (Nthiwa *et al.*, 2019). Whereas the establishment of wildlife conservancies provides a sustainable way of integrating wildlife conservation

alongside livestock production (Løvschal *et al.*, 2019), it also intensifies livestock-wildlife interactions which may increase infectious disease transmission (Nthiwa *et al.*, 2019).

This study investigated how different land use types affect disease exposure among cattle herds raised in the Mara ecosystem, using *Leptospira* spp. and *Brucella* spp. as case study pathogens. Specifically, this study determined the seroprevalence of these pathogens in cattle across three zones with varying levels of wildlife-livestock interactions and identified risk factors associated with exposure. This study provides information on the current epidemiological situation of these pathogens in the area. It will also provide additional data to inform discussions on the linkages between host diversity and infectious disease risk.

4.3 Materials and methods

4.3.1 Study area

The study area and compliance with ethical standards are described in **chapter 3** (section 3.3.1 and 3.3.2, respectively). Three ecological zones were identified along a transect from the Maasai Mara National Reserve (MMNR) reserve to inhabited areas, representing variations in land use patterns, from extensively raised large livestock herds and no crop production nearer the reserve to mixed farming (livestock production and crop cultivation) in areas further away from the reserve. The immediate areas bordering the reserve and wildlife conservancies constituted zone 1 (“high interface area”), characterized by intense wildlife-livestock interactions. Zone 2 (“moderate interface area”) was represented by areas 20 to 40 km away from the reserve, with moderate wildlife-livestock interactions; while zone 3 (“low interface area”) was the area more than 40 km away from the reserve, where wildlife-livestock

interactions are more rare (Ogutu *et al.*, 2009; Bhola *et al.*, 2012). These defined ecological zones allowed the analysis of risk factors associated with *Leptospira* spp. and *Brucella* spp. seroprevalence to be compared across the various zones with different levels of wildlife-livestock interactions and varied land use types.

4.3.2 Selection of villages

Five villages across the zones were purposely selected following participatory consultations with local communities to classify villages based on wildlife-livestock interactions. Two villages were selected in zone 1 (Mara Rianta and Oololaimutia), another two in zone 2 (Lemek and Endoinyio Narasha) and one in zone 3 (Nkorinkori) (Figure 4.1). The selected villages had similar characteristics as those of the respective zones.

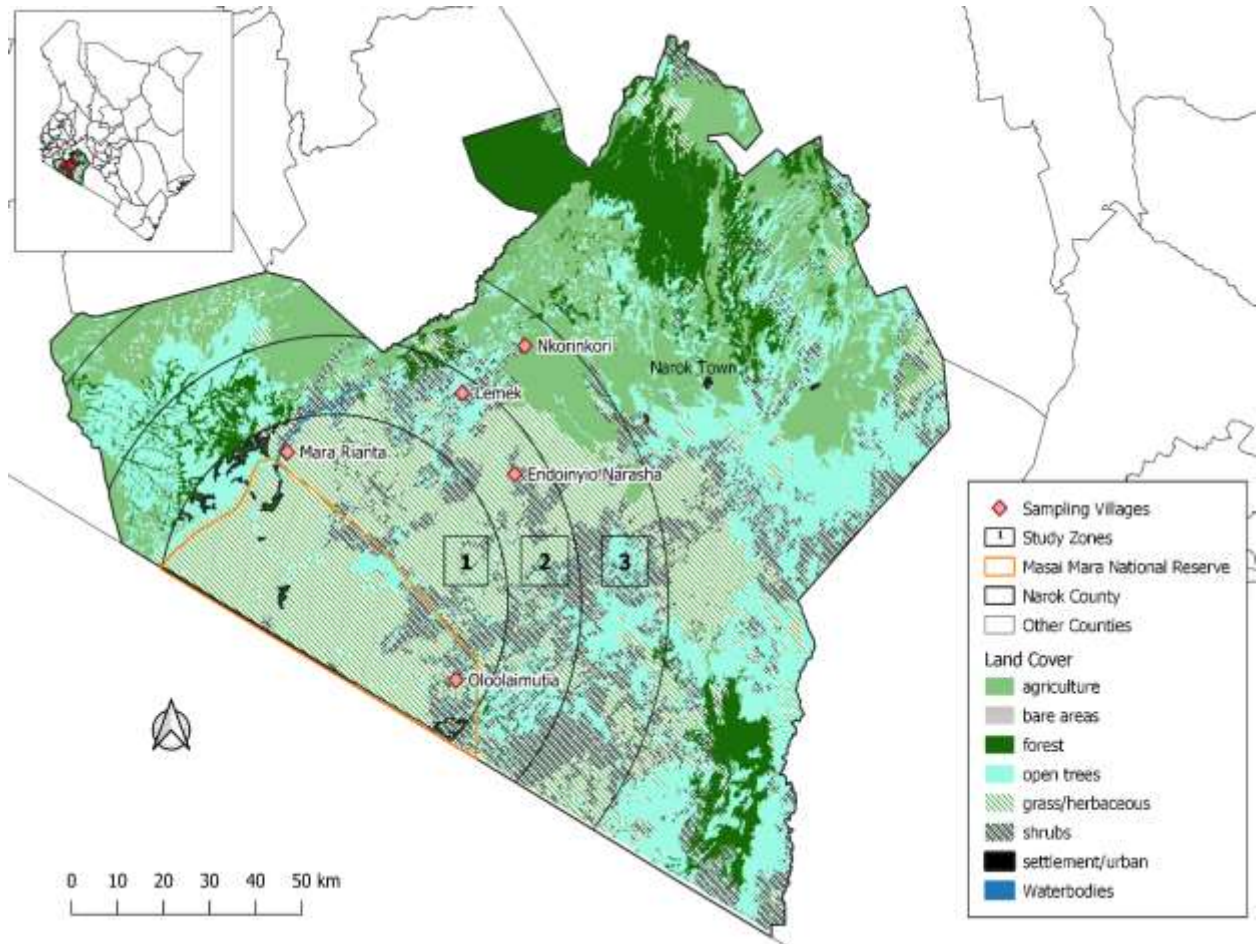


Figure 4.1: Location of sampling sites within the Maasai Mara ecosystem

4.3.3 Study design, sample size estimation and collection of epidemiological data

A cross-sectional study with multistage cluster sampling was conducted between September 2016 and July 2017. The total number of animals sampled per zone was estimated using the formula; $n = (1.96)^2 p(1-p)/d^2$, with a margin error (d) of 0.05 (Dohoo *et al.*, 2012). In the absence of previous information on disease prevalence in the area, this study assumed a seroprevalence (p) of 50% for both diseases. To account

for the design effect (variance inflation factor) due to clustering of cattle in herds, the initial sample size was adjusted using the formula; $n^1 = n(1+\rho(m-1))$, where n^1 is the new sample size, ρ (*rho*) is the Intra-cluster (intra-herd) Correlation Coefficient (ICC) and m is the number of animals to be sampled per herd (Dohoo *et al.*, 2012). An ICC of 0.1 was used for both diseases and was informed by other studies conducted elsewhere (Segura-Correa *et al.*, 2010; Kanouté *et al.*, 2017), given the limited information on this parameter in the study area. In this study, 3 randomly-selected animals were sampled in each herd. The adjusted sample size was 465 cattle (from 155 herds) per zone. The study used probability proportional to herd size sampling method to sample herds within zones. A total of 465 cattle was sampled each from zones 1 and 2 (both with many cattle herds) while in zone 3 (with limited number of herds), a total of 240 cattle (from 80 herds) were sampled. In each village, livestock-keeping households were randomly selected from a household list prepared with the assistance of the area chiefs. In each selected household, the herd found in the village at the time of visit was sampled (as households could own more than one herd). The study targeted animals aged ≥ 1 year as these are the animals that interact with animals from other herds during grazing or sharing of water sources (Nthiwa *et al.*, 2019), given that younger animals are normally kept in the farm area and not taken for grazing. Animals aged more than one year were therefore expected to have a higher relative risk of infectious disease exposure compared to calves. These animals also travelled longer distances than young ones, and could be used more reliably for the surveillance of both diseases in the area.

A questionnaire was administered in each household to collect epidemiological data on putative risk factors for transmission of brucellosis and leptospirosis in cattle. At the animal-level, information was collected on animal sex and age. At herd-level, data

was collected on herd size (number of cattle belonging to the household at the time of sampling), history of abortions, herd management practices (sedentary or semi-nomadic pastoralism), source of breeding bull, grazing strategies, watering sources and purchase of livestock in the past year (yes or no). The questionnaire is provided as appendix 3.

4.3.4 Sample collection and processing

From each animal, 10 ml jugular blood was collected into plain vacutainer tubes labelled with unique barcodes. The samples were stored in cool boxes at +4°C and at the end of the day, they were transported to the Kenya Wildlife Service's (KWS) field laboratory facility in the Maasai Mara. To extract sera, the clotted blood samples were centrifuged at 3000 g (gravitational force) for 6 minutes and the obtained sera aliquoted into two 2 ml uniquely barcoded cryovials (Thermo Fisher Scientific). Sera samples were stored at -20°C until further processing at the Biosciences laboratory facilities of the International Livestock Research Institute (ILRI), Nairobi.

4.3.5 Serological testing

4.3.5.1 *Brucella* spp. antibody test

Testing for antibodies (IgG1) against *Brucella abortus* was done using a commercially available indirect ELISA kit (PrioCHECK® *Brucella* Antibody 2.0 indirect ELISA kit, Prionics AG, Netherlands) following the manufacturer's instructions. The positive and negative reference sera were run in duplicates while samples were tested in singles for each test plate. The optical densities (ODs) of samples were measured at 450 nm using a microplate reader (BioTek® Winooski, VT, USA) and expressed as relative OD by dividing the OD₄₅₀ of test samples by the mean OD₄₅₀ of positive controls and

multiplying the result by 100. As recommended by the manufacturer, animals were classified as negative if the relative OD was $\leq 40\%$ and positive if $>40\%$.

4.3.5.2 *Leptospira* spp. antibody test

The detection of antibodies against *Leptospira interrogans* serovar *hardjo* was also done using a commercially available kit from Prionics, AG, Netherlands (PrioCHECK® *L. hardjo* indirect ELISA) and following the manufacturer's instructions. In brief, the test samples, reference sera (positive, negative and weak positive controls) and blank controls were run in duplicates for each test plate. The ODs of the tested samples were read at 450 nm using a microplate reader. To interpret test sample ODs, the corrected OD₄₅₀ values of the test samples and positive controls were first obtained by subtracting the mean OD₄₅₀ of the blanks from each. The relative OD of tested sera was then calculated using the formula;

$$\% \text{ positivity} = \frac{\text{corrected OD}_{450} \text{ of test sample}}{\text{corrected OD}_{450} \text{ of positive control}} \times 100\%$$

Animals were classified as negative if the percentage positivity was $<20\%$, inconclusive if between 20-45% and positive if $>45\%$. Sera samples with inconclusive antibody titers were retested and if unresolved, they were included as negatives in the data analysis.

4.3.6 Data analyses

Questionnaire and serological data were entered in MS Excel (Microsoft® Excel, Washington, 2013) and analysis was done using R software, version 3.3.3 (R Core Team, 2019). Descriptive analyses including the calculation of seroprevalence and 95% confidence intervals were done using the packages *DescTools* (Signorell *et al.*, 2016) and *gmodels* (Warnes *et al.*, 2009). Animal sex and zone were independently

assessed for their association with *Brucella* spp. or *Leptospira* spp. seroprevalence using χ^2 test.

Risk factor analysis was done at animal- and herd-levels. A herd was classified as seropositive for either *Brucella* spp. or *Leptospira* spp. if one or more animals within the herd tested positive in the respective ELISA. The investigated risk factors were first tested for their association with animal- and herd-level seropositivity of both diseases, using univariable logistic regression models. Causal diagrams (i.e., Directed Acyclic Graphs, DAGs) (Joffe *et al.*, 2012) were constructed for significant predictors ($p < 0.05$) in the univariable analyses to select variables for multivariable analyses using generalized linear mixed-effects models (GLMM). Both univariable and multivariable analyses were done using the *glmer* function of the *lme4* package (Bates *et al.*, 2014), with adjustment for herd clustering (herd ID as a random effect) in the animal-level models and for village-level clustering (village ID as a random effect) in the herd-level models. The variable representing zones was forced as a fixed effect in the GLMM analyses. The analysis used a forward-backward stepwise procedure to select the final models. In the first step, a full model was fitted with the selected variables from the univariable analyses and removed those with $p > 0.05$ based on the Wald χ^2 test. Thereafter, the removed variables were re-entered one by one (those with the smallest p-value were added first) and dropped if the p-value was > 0.05 . The final models were selected based on the lowest Akaike Information Criterion (AIC). The covariates in the final model were assessed for potential interaction effects using pairwise-factor product terms and tested for main effects using the likelihood ratio test (LRT). Model fit was tested using the Hosmer-Lemeshow goodness of fit test, while

the ICCs for herd- and village-levels clustering were calculated using the `icc` function of `sjstats` package (Lüdtke, 2017).

4.4 Results

4.4.1 Descriptive results

Blood samples were obtained from 1,170 cattle (21.4% and 78.6% males and females respectively) belonging to 390 herds. The median cattle herd size was 50 (range; 4-570). The overall apparent animal-level seroprevalence of *Brucella* spp. and *Leptospira* spp. were 36.9% (95% CI; 34.1-39.8) and 23.5% (95% CI; 21.1-26.0) respectively (Table 4.1). Animal-level seroprevalence of both diseases differed between zones; *Brucella* spp. seroprevalence was higher in zone 1 (high interface area) than in zones 2 and 3 ($\chi^2 = 25.1$, $df = 2$, $p < 0.001$). Zones 1 and 2 had significantly higher *Leptospira* spp. seroprevalence than zone 3 ($\chi^2 = 7.0$, $df = 2$, $p = 0.029$). Overall, the level of *Brucella* spp. and *Leptospira* spp. co-exposure in animals was estimated at 8.8% (95% CI; 7.3-10.4) and differed significantly by sex ($\chi^2 = 9.9$, $df = 1$, $p = 0.001$) with females having higher levels of co-exposure (10.2%; 95% CI; 8.4-12.1) than males (3.6%; 95% CI 1.6-5.6). There were no differences in the levels of co-exposure between zones ($p > 0.05$).

At herd-level, 68.7% (95% CI; 66.1-71.5) of the herds had at least one seropositive animal for *Brucella* spp. and 52.7% (95% 49.7-55.6) had at least one animal positive for *Leptospira* spp. (Table 4.1). The herd-level seroprevalence of both diseases varied significantly by zones ($p < 0.001$), following a similar pattern as that of animal-level seroprevalence mentioned above. Herd-level seroprevalence of brucellosis was higher in zone 1 than other zones while for leptospirosis, zones 1 and 2 had a significantly

higher seroprevalence than zone 3. The spatial distributions of *Brucella* spp. and *Leptospira* spp. seropositive herds are presented in Figure 4.2.

Table 4.1: Animal- and herd-level apparent seroprevalences of *Brucella* spp. and *Leptospira* spp. and the levels of co-exposure in various zones

	High interface area (Zone 1)		Moderate interface area (Zone 2)		Low interface area (Zone 3)		Overall seroprevalence
	No. tested (n)	% seropositive (95% CI)	No. tested (n)	% seropositive (95% CI)	No. tested (n)	% seropositive (95% CI)	% seropositive (95% CI)
<i>Brucella</i> spp.							
Animal-level	465	45.6 (41.1-50.4)	465	31.8 (27.7-36.3)	240	30.0 (24.6-36.2)	36.9 (34.1-39.8)
Herd-level	155	81.3 (77.8-84.8)	155	60.0 (55.5-64.7)	80	61.3 (55.0-67.5)	68.7 (66.1-71.5)
<i>Leptospira</i> spp.							
Animal-level	465	24.7 (20.9-28.7)	465	25.6 (21.7-29.7)	240	17.1 (12.0-22.1)	23.5 (21.1-26.0)
Herd-level	155	52.9 (48.4-57.8)	155	57.4 (53.0-62.2)	80	42.5 (36.3-49.0)	52.7 (49.7-55.6)
Co-exposure	465	10.8 (8.2-13.5)	465	8.4 (6.2-11.0)	240	5.8 (3.3-8.7)	8.8 (7.3 - 10.4)

CI, confidence interval; n at animal-level is the number of animals tested while at herd-level, it refers to number of herds tested.

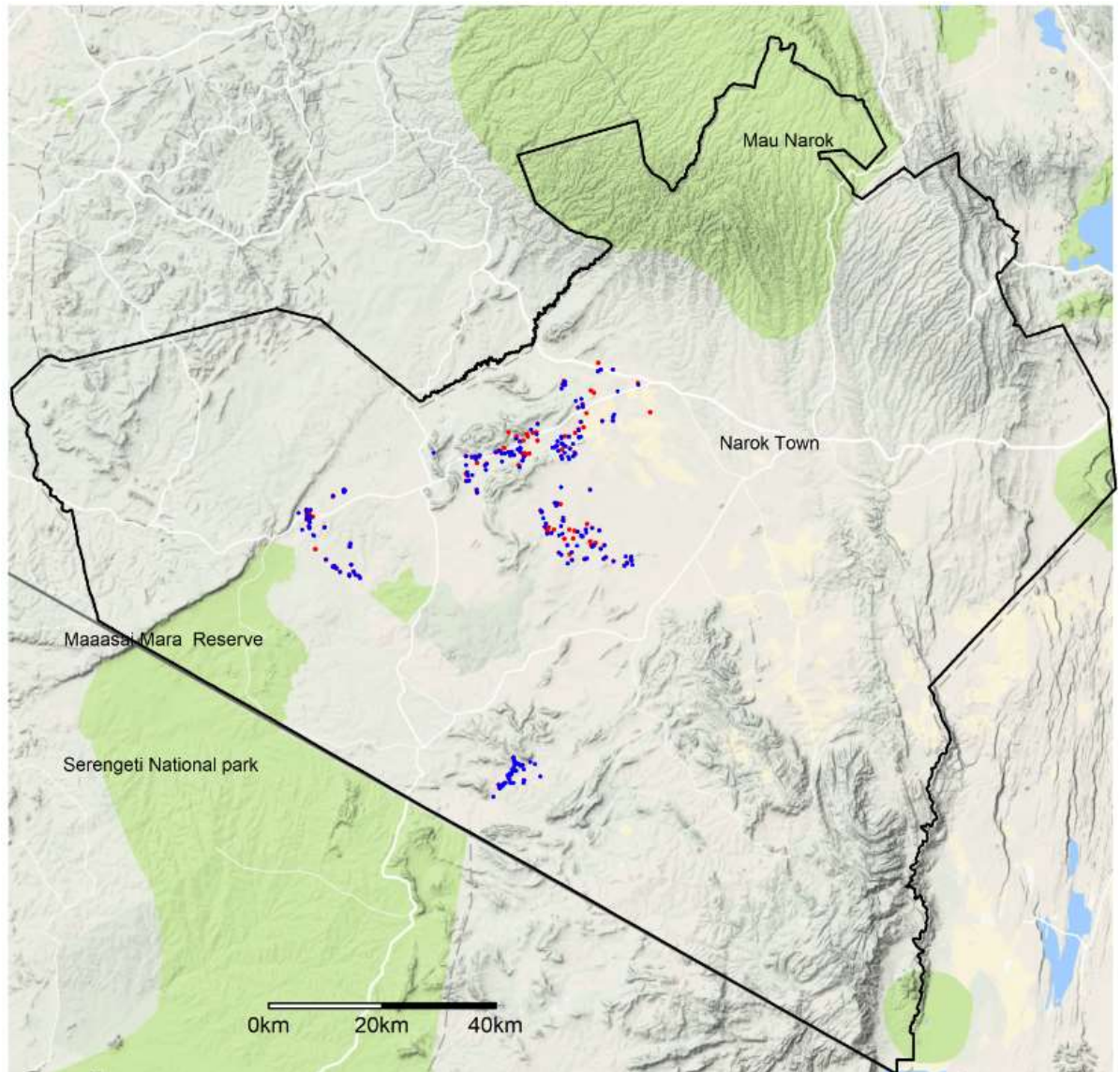


Figure 4.2: Spatial distribution of *Brucella* spp. (in blue dots) and *Leptospira* spp. (in red dots) seropositive herds in various ecological zones with different levels of wildlife-livestock interactions.

4.4.2 Risk factors associated with *Brucella* spp. seropositivity

Table 4.2 shows variables found to be statistically significantly associated with animal-level seroprevalence of *Brucella* spp. and *Leptospira* spp. (with adjustment for herd-level clustering). For both diseases, animal sex ($p < 0.001$) was a significant predictor of animal-level seroprevalence, with more females being exposed than males. In the

case of *Brucella* spp., raising of cattle in areas with intense wildlife-livestock interactions (zone 1), utilizing of watering points shared between villages, mixing of cattle with others (from a different herd) during grazing, management of cattle under pastoral systems and grazing in the wildlife reserves, were all identified as significant predictors ($p < 0.05$) in the univariable analyses.

Table 4.2: Risk factors associated with animal-level seroprevalence of *Brucella* spp. and *Leptospira* spp. based on univariable logistic regression with a random effect for herd

Variable and category	<i>Brucella</i> spp.				<i>Leptospira</i> spp.			
	No. tested	% prevalence (95% CI)	Odds ratio (95% CI)	P-value	No. tested	% prevalence (95% CI)	Odds ratio (95% CI)	P-value
Animal sex								
Male	250	24.4 (19.2 - 29.7)	1 (Ref.)		250	15.6 (11.6 - 20.2)	1 (Ref.)	
Female	920	40.3 (37.1 - 43.6)	2.5 (1.7 - 3.7)	< 0.001	920	25.7 (22.8 - 28.5)	1.9 (1.3 - 2.8)	0.001
Study zones								
Low interface area (zone 3)	240	30.0 (24.6 - 36.2)	1 (Ref.)		240	17.1 (12.0-22.1)	1 (Ref.)	
Moderate interface area (zone 2)	465	31.8 (27.7 - 36.3)	1.1 (0.7 - 1.6)	0.657	465	25.6 (21.7-29.7)	1.7 (1.1 - 2.6)	0.015
High interface area (zone 1)	465	45.6 (41.1 - 50.4)	2.1 (1.4 - 3.1)	< 0.001	465	24.7 (20.9-28.7)	1.6 (1.1 - 2.5)	0.028
History of abortions in the surveyed herds								
No	587	22.7 (19.4 - 26.2)	1 (Ref.)		587	20.5 (17.4 - 23.8)	1 (Ref.)	
Yes	583	24.4 (20.9 - 27.9)	1.1 (0.8 - 1.5)	0.458	583	26.5 (22.9 - 30.1)	1.4 (1.1 - 2.0)	0.019
Shared watering points between villages								
No	510	32.0 (28.0 - 36.3)	1 (Ref.)		510	22.2 (18.6 - 25.8)	1 (Ref.)	
Yes	660	40.8 (37.0 - 44.7)	1.5 (1.1 - 2.1)	0.005	660	24.5 (21.4 - 27.9)	1.2 (0.8 - 1.6)	0.366
Mix cattle with others herd during grazing								
No	177	29.4 (23.2 - 36.5)	1 (Ref.)		177	19.8 (14.7 - 26.0)	1 (Ref.)	
Yes	993	38.3 (35.1 - 41.4)	1.6 (1.0 - 2.4)	0.042	993	24.2 (21.6 - 26.9)	1.3 (0.8 - 2.0)	0.228
grazing areas shared between villages								
No	873	35.9 (32.6 - 39.2)	1 (Ref.)		873	21.5 (18.9 - 24.3)	1 (Ref.)	
Yes	297	40.0 (34.7 - 46.1)	1.2 (0.9 - 1.7)	0.244	297	29.3 (24.2 - 34.6)	1.5 (1.1 - 2.2)	0.011
Herd management practice								
Sedentary	660	32.0 (28.5 - 35.7)	1 (Ref.)		660	21.0 (17.9 - 24.0)	1 (Ref.)	
Pastoral	510	43.3 (39.0 - 47.9)	1.7 (1.3 - 2.3)	< 0.001	510	26.9 (23.1 - 30.8)	1.4 (1.0 - 1.9)	0.025

Grazing of cattle in wildlife reserves									
	No	666	32.3 (28.7 – 36.0)	1 (Ref.)		666	21.3 (18.3 – 24.5)	1 (Ref.)	
	Yes	504	43.1 (38.7 - 47.6)	1.7 (1.2 - 2.3)	< 0.001	504	26.4 (22.6 – 30.3)	1.3 (1.0 - 1.8)	0.056
Utilize a communal grazing area									
	No	858	36.2 (33.0 - 39.6)	1 (Ref.)		858	20.7 (18.1 – 23.4)	1 (Ref.)	
	Yes	312	38.8 (33.3 - 66.8)	1.1 (0.8 - 1.6)	0.478	312	31.1 (26.0– 36.3)	1.8 (1.3 - 2.5)	<0.001
Herd size									
	≤ 49 cattle	498	36.0 (31.7 - 40.4)	1 (Ref.)		498	20.5 (17.1 - 24.1)	1 (Ref.)	
	≥ 50 cattle	672	37.6 (33.9 - 41.4)	1.1 (0.8 - 1.5)	0.624	672	25.7 (22.5 – 29.1)	1.4 (1.1 – 1.90)	0.046

Ref, reference category; CI, lower and upper limits for 95% confidence intervals.

The results of univariable analyses for herd-level risk factors of *Brucella* spp. and *Leptospira* spp. (with adjustment for village-level clustering) are presented in Table 4.3. There was a significant association ($p < 0.05$) of herd-level seroprevalence of *Brucella* spp. with previous purchase of livestock, grazing in areas shared between villages and cattle utilizing a communal grazing reserve.

Table 4.3: Risk factors associated with herd-level seroprevalence of *Brucella* spp. and *Leptospira* spp. based on univariable logistic regression with a random effect for village.

Variables	Category	Odds ratio (95% CI)	P-value
1. Brucellosis			
Previous purchase of livestock	No	1 (Ref.)	0.031
	Yes	1.4 (1.0-1.8)	
Grazing in areas shared between villages	No	1 (Ref.)	0.005
	Yes	2.0 (1.2-3.3)	
Utilizing a communal grazing reserve	No	1 (Ref.)	0.012
	Yes	2.0 (1.2-3.5)	
2. Leptospirosis			
Mix cattle with others (from a different herd) at watering points	No	1 (Ref.)	0.026
	Yes	1.4 (1.0-1.9)	
Herd management practice	Sedentary	1 (Ref.)	0.010
	Pastoral	1.8 (1.1-2.7)	
Grazing of cattle in wildlife reserves	No	1 (Ref.)	0.038
	Yes	1.5 (1.0-2.2)	
Herd size	≤ 49 cattle	1 (Ref.)	0.012
	≥ 50 cattle	1.4 (1.1-1.7)	

Ref, reference category; CI, lower and upper limits for 95% confidence intervals.

The results of multivariable analysis showed cattle sex (female) and zones (high interface area) as important predictors of animal-level seropositivity of *Brucella* spp. (Table 4.4). The multivariable model fitted for herd-level risk factors identified purchase of livestock and herds utilizing shared grazing areas between villages, as significant risk factors for herd-level brucellosis seropositivity (Table 4.5). From the variance components of these models, the estimated ICCs for herd (i.e., the level of dependence among cattle individuals within herd) and village (i.e., the level of dependence among herds of the same village) were respectively, 0.16 (95% CI; 0.07-0.24) and 0.18 (95% CI; 0.01-0.34) for *Brucella* spp.

Table 4.4: Final models of animal-level risk factors for *Brucella* spp. and *Leptospira* spp. in cattle based on GLMM analysis

Variables	Category	Odds ratio (95% CI)	P-value
1. Brucellosis*			
Fixed effects			
Animal sex			
	Male	1 (Ref.)	
	Female	2.8 (1.9 - 4.1)	< 0.001
Study zones			
	Low interface area (zone 3)	1 (Ref.)	
	Moderate interface area (zone 2)	1.2 (0.8 - 1.8)	0.490
	High interface area (zone 1)	2.5 (1.7 - 3.9)	< 0.001
2. Leptospirosis*			
Fixed effects			
Animal sex			
	Male	1 (Ref.)	
	Female	2.1 (1.4 - 3.1)	< 0.001
Study zones			
	Low interface area	1 (Ref.)	
	Moderate interface area	1.6 (1.0 - 2.5)	0.034
	High interface area	1.3 (0.8 - 2.1)	0.302
Utilizing of communal grazing reserve			
	No	1 (Ref.)	
	Yes	1.9 (1.3 - 2.7)	0.001

Ref, reference category; CI, lower and upper limits for 95% confidence intervals.

*The random variable (i.e., herd ID) used to account for the clustering of brucellosis and leptospirosis within herds were 0.59 and 0.22 respectively.

Table 4.5: Final models of herd-level risk factors for *Brucella* spp. and *Leptospira* spp. in cattle GLMM analysis

Variables	Category	Odds ratio (95% CI)	P-value
1. Brucellosis*			
Fixed effects			
Study zones	Low interface area (zone 3)	1 (Ref.)	
	Moderate interface area (zone 2)	0.8 (0.1 - 5.3)	0.854
	High interface area (zone 1)	2.5 (0.4 - 16.4)	0.969
Purchase of livestock in the previous year	No	1 (Ref.)	
	Yes	1.4 (1.0 - 1.9)	0.024
Share grazing areas between villages	No	1 (Ref.)	
	Yes	1.9 (1.2 - 3.2)	0.012
2. Leptospirosis*			
Fixed effects			
Study zones	Low interface area	1 (Ref.)	
	Moderate interface area	1.6 (0.8 - 3.5)	0.200
	High interface area	0.8 (0.3 - 1.9)	0.626
Herd management practice	Sedentary	1 (Ref.)	
	Pastoral	1.9 (1.2 - 3.1)	0.010
Herd size	≤ 49 cattle	1 (Ref.)	
	≥ 50 cattle	1.3 (1.0 - 1.7)	0.035

Ref, reference category; CI, lower and upper limits for 95% confidence intervals.

*The random variable (i.e., village ID) used to account for the clustering of brucellosis and leptospirosis within villages were 0.57 and 0.08 respectively.

4.4.3 Risk factors associated with *Leptospira* spp. seropositivity

The univariable models for animal-level risk factors of *Leptospira* spp., identified the raising of cattle in areas with moderate and high wildlife-livestock interactions; positive history of abortions in the surveyed herds, grazing of cattle in areas shared between villages, management of cattle under pastoral systems, grazing in the wildlife reserves, cattle utilizing a communal grazing reserve and herd size with ≥ 50 animals, as significantly associated ($p < 0.05$) with animal-level *Leptospira* spp. seropositivity (Table 4.2).

The most important herd-level risk factors for *Leptospira* spp. (based on univariable analyses) included; mixing of cattle with others (from a different herd) at watering points, pastoral herd management practice, grazing in wildlife reserves and herd size with ≥ 50 animals (Table 4.3).

The multivariable model identified cattle sex (female), zones (moderate interface area) and utilizing a communal grazing reserve as significant predictors of animal-level seropositivity of *Leptospira* spp. (Table 4.4). At herd-level, the final multivariable model showed that pastoral herd management practice and herd size with ≥ 50 animals were significant predictors of herd-level seropositivity of *Leptospira* spp. (Table 4.5). The estimated ICCs for herd- and village-level clustering were 0.10 (95% CI; 0.00-0.19) and 0.04 (95% CI; 0.00-0.10) respectively for *Leptospira* spp.

The assessment of pairwise-factor product terms of the covariates in the final models did not show significant interaction effects ($p > 0.05$) and no confounders were detected. The Lesmer-Hosmer goodness of fit test also indicated that the final models fitted the data well ($p > 0.05$).

4.5 Discussion

This study determined the association between *Brucella* spp. and *Leptospira* spp. seroprevalence with land use patterns in the Mara ecosystem, Kenya. Both *Brucella* spp. and *Leptospira* spp. were found to be prevalent in the area as evidenced by the high levels of exposure at animal- and herd-levels. The animal-level seroprevalence of *Brucella* spp. found in this study (36.9%) was higher compared to 12.44% (n = 225) previously reported in the area (Enström *et al.*, 2017), but was aligned with the findings of Nina *et al.* (2017) in Uganda (44%) and Madut *et al.* (2018) in Sudan (31%). *Leptospira* spp. animal-level seroprevalence was also consistent with earlier reports in resource-poor areas, for instance, 25-34% in Kenya (de Vries *et al.*, 2014) and 22.2% in Lao People's Democratic Republic (Olmo *et al.*, 2018). The high seroprevalences of these pathogens are worrying as both diseases are well known threats to animal productivity. Both diseases cause abortion and reduced productivity. They are also highly infectious and hard to eradicate from a herd or area without active interventions to identify, treat and/or remove infected animals. Vaccination of livestock against brucellosis (Njeru *et al.*, 2016) and leptospirosis is rarely done in the region and in Kenya, and therefore the high seroprevalences found for these pathogens are likely due to natural exposure. The ELISA kits used are also not perfect as the specificity of both kits have been estimated to range from 98 to 100% for *Brucella* spp. (Tschopp *et al.*, 2015) and to be about 85% for *Leptospira* spp. (Barrett *et al.*, 2018). The lack of 100% specificity in both ELISA tests may have yielded low rates of false positives. Nevertheless, these high seroprevalences are aligned with previous studies in the region. For example, 35.6% for *Leptospira* spp. in Kenya (Rajeev *et al.*, 2017) and 7.5 to 40% for *Brucella* spp. in various pastoral production systems across Africa (McDermott & Arimi, 2002).

While this study did not assess humans' exposure to *Brucella* spp. or *Leptospira* spp., the communities in the surveyed zones may be at risk of zoonotic infection through food (e.g., raw milk consumption) and occupational exposure. Indeed, an earlier study conducted in the area reported a seroprevalence of 21.2% (range; 13.8 – 35.9) among hospital patients with flu-like symptoms (Muriuki *et al.*, 1997). A recent study conducted in the area also reported clinical signs that are compatible with brucellosis in cattle and among animals handlers (Enström *et al.*, 2017).

In the analyses, this study accounted for clustering of cattle within herds and villages using herd and village IDs as random effects, respectively. The Intra-cluster (intra-herd) Correlation Coefficients (ICCs) for both diseases was found to be moderate. This is likely due to cattle within herds sharing a common environment (i.e., common grazing, shared water sources) and similar management practices (Segura-Correa *et al.*, 2010). With respect to villages, there was a substantial clustering of herd-level brucellosis while for *Leptospira* spp., the low ICC indicated lack of village-level clustering (i.e., herd-level exposure was independent of villages).

The acquisition of new animals through purchase was an important risk factor for herd-level brucellosis, in agreement with earlier reports in Uganda (Bugeza *et al.*, 2018). This finding could be due to the likelihood of introducing infected animals into a healthy herd if the health status of sourced animals is not determined or temporally quarantine is not enforced. Further studies should clarify how herd dynamics due to livestock offtake or purchase can influence the prevalence of *Brucella* spp. in the area. *Brucella* spp. seroprevalence was also higher as the sites got closer to the Mara reserve. This finding may be associated with the different land use types adopted in the surveyed zones. Land use changes are thought to modify the interactions between host

species and thus can directly or indirectly influence the level of pathogen transmission between hosts (Patz *et al.*, 2004; Gottdenker *et al.*, 2014). In zone 1 for example, cattle are raised in extensive systems as farmers utilize wildlife conservancies and MMNR compared to zones 2 and 3 with sedentary and crop-livestock mixed agriculture, respectively. Extensive livestock production systems (e.g., pastoralism) allows multiple herds to share common grazing and watering points which may increase chances of naive cattle encountering infected or carrier state animals including wildlife (McDermott & Arimi, 2002). Indeed this study identified pastoral husbandry practice as a significant predictor of both brucellosis and leptospirosis seropositivity in cattle. For *Leptospira* spp., seroprevalence in zones 1 and 2 differed significantly with zone 3 (low interface area), but not between zones 1 and 2. The lack of significant differences in seroprevalence between zones 1 and 2 indicated that variations in land use patterns between the two zones alone, may be inadequate to show exposure difference for this pathogen in cattle.

The higher seroprevalence of *Brucella* spp. in zone 1 compared to zones 2 and 3 could also be partly due to the likelihood of high interactions between wildlife and livestock, given that these animals graze within the MMNR. Although the biological sampling of wildlife was not conducted in the study, the interactions between wildlife and livestock in the area is a possible factor that could also account for the differences in seroprevalence of this pathogen in the zones. Zone 1, for example, has a higher diversity of wildlife species (i.e., wildlife host species richness) than zone 3, which could increase infectious disease transmission as it may create a large pool of pathogen reservoirs (Daszak *et al.*, 2000; Keesing *et al.*, 2010) including *Brucella* spp. which is shared with cattle (Godfroid, 2018). Information on brucellosis (Njeru *et al.*, 2016)

and leptospirosis in wildlife species is very limited in the area and indeed in Kenya, but *Brucella* spp. exposure in various wildlife species including the African buffalo (*Syncerus caffer*) and blue wildebeest (*Connochaetes taurinus*) has been documented in the Mara ecosystem (Waghela & Karstad, 1986). Besides wildlife, rodents are also important sources of various *Leptospira* species (Allan *et al.*, 2015) and can contaminate grazing areas or watering resources utilized by livestock. Both *Leptospira* spp. and *Brucella* spp. are known to persist in the environment with survival duration being affected by factors such as ultra violet (UV) light, pH, salinity, soil moisture and temperature (Estrada-Peña *et al.*, 2014). The persistence of *Brucella* spp. in water and soil may range between 21 and 81 days (Aune *et al.*, 2012), while for *Leptospira* spp., it can vary from hours to 193 days (Casanovas-Massana *et al.*, 2018). The ability of these pathogens to persist in water or grazing areas can influence the indirect transmission processes of zoonotic diseases if these resources are contaminated with infected excreta or urine (Mwachui *et al.*, 2015). Sharing of these ecological resources by different livestock herds may also promote direct transmission of zoonotic diseases through increased intra- and inter-herd interactions (Rajeev *et al.*, 2017). Indeed, this study found common utilization of watering points, grazing areas or mixing of cattle herds at these key resources as important predictors of brucellosis and leptospirosis seropositivity in cattle. Whereas the role played by small ruminants (sheep and goats) in the epidemiology of *Brucella* spp. and *Leptospira* spp. in the area is largely unknown, the interactions between cattle and small ruminants may also increase the inter-species transmission levels of these pathogens. Small ruminants are increasingly becoming important sources of household livelihoods in the area (Løvschal *et al.*, 2019; Nthiwa *et al.*, 2019), and their population densities is estimated to have increased by 235.6% between 1977 and 2014 compared to cattle populations by 0.8% between

same period (Bedelian & Ogutu, 2017). The high population densities of small ruminants in the area may also create a large pool of maintenance hosts for these pathogens.

This study found higher seroprevalences of *Brucella* spp. and *Leptospira* spp. among female cattle than males. In general, cows have lower offtake rates than bulls in Maasai Mara ecosystem as they are raised to provide milk, an important diet for the locals (Nthiwa *et al.*, 2019), and also for breeding purposes to replace animals that may die due to recurrent droughts (Huho *et al.*, 2011). As cows stay in herds longer than bulls, they could have high chances of repeated exposure to these pathogens over time. The high proportion of exposed females also presents a major risk of transmission to male populations through natural breeding which is predominant in the surveyed zones.

The finding that positive history of abortions among surveyed herds was associated with animal-level leptospirosis, could be due to poor husbandry practices such as improper disposal of aborted fetuses and placenta, resulting to environmental contamination (Mwachui *et al.*, 2015). Aborting animals retained in the herds may also act as sources of infections with subsequent parturitions through uterine discharges (Loureiro *et al.*, 2017). Although abortions in cattle are caused by many diseases (e.g., foot and mouth disease, bovine trypanosomiasis and contagious bovine pleuropneumonia), the results suggest that *Leptospira* spp. could be one of the major causes in the area and further studies should clarify this finding. The positive association between large herd sizes (≥ 50 animals) and *Leptospira* spp. exposure of animals may be due to greater animal contacts within larger herds (Barrett *et al.*, 2018). The management of large herds also involves frequent movements in search of water

and pasture, more so in dry season. This practice may contribute to the spread of infectious diseases but may also expose herds to diseases that may be limited to an area (Alhaji *et al.*, 2016).

4.6 Limitations

This study aimed at investigating how cattle-herd distance to wildlife reserves in Kenya may affect the prevalence of two major animal infectious diseases. Such potential effect could derive from cattle interactions with wildlife, or by farm management characteristics that relate to the herd's location in relation to the MMNR (i.e. land-use). This study did not sample wildlife to determine their exposure status with regard to the target pathogens and therefore the role of wildlife in the observed seroprevalence cannot be confirmed and thus these observations on this regard remain speculative. There are also drawbacks related to the serological tests used to determine the seroprevalences of *Brucella* spp. and *Leptospira* spp. in cattle. For instance, animal's seropositivity to any of these pathogens indicates exposure and does not imply that the animal had active or current infections at the time of sampling. The PrioCHECK[®] *Brucella* Antibody indirect ELISA kit was used to test for antibodies against *Brucella* spp. in cattle but there is known cross-reactivity between anti-lipopolysaccharides of *Brucella abortus* and those of other Gram-negative bacteria such as *Francisella tularensis*, *Campylobacter* spp., *Salmonella* spp., *Pasteurella* spp., *Yersinia enterocolitica* 0:9, *Escherichia coli* O:117 and O:156 thus potentially yielding false positives (Bonfini *et al.*, 2018). The testing for antibodies against *Leptospira* spp. was also performed using PrioCHECK[®] *L. hardjo* indirect ELISA kit rather than the microscopic agglutination test (MAT) which is considered the reference test (Adler & de la Peña Moctezuma, 2010). Therefore, it is possible that the reported seroprevalence

rates are an overestimation of the true rates. The study used a cross-sectional study design and it was not possible to explore how the incidence patterns of these pathogens may vary over time.

4.7 Conclusion

This study provides data on the current epidemiological situation of *Brucella* spp. and *Leptospira* spp. exposure in cattle herds raised in the Mara ecosystem. The findings of this study demonstrated that both diseases are prevalent in the area and had a considerable level of co-exposure in animals. Seroprevalence of *Brucella* spp. was higher in areas near Mara reserve (zone 1) compared to other zones. For *Leptospira* spp., zones 1 and 2 had significantly higher seroprevalence than zone 3. The seropositivity of both diseases was also significantly associated with grazing cattle in wildlife reserves. As these pathogens could spillover from wildlife reservoirs into livestock in areas with close interactions, further studies are needed to establish exposure levels in wildlife, sheep and goats, and humans. Furthermore, mapping the transmission routes of these pathogens and quantifying their impacts on cattle production will help in the development of appropriate control strategies.

CHAPTER FIVE

5.0 SEROPREVALENCE AND RISK FACTORS OF FOOT AND MOUTH DISEASE VIRUS IN CATTLE AT THE WILDLIFE-LIVESTOCK INTERFACE AREA IN MAASAI MARA, KENYA

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5.1 Abstract

A cross-sectional study was carried out to determine foot and mouth disease (FMD) seroprevalence and identify risk factors of exposure in cattle across three zones with different land use types and varying levels of wildlife-livestock interactions. Five villages were selected purposively; two in zone 1 with intense wildlife-livestock interactions, another two in zone 2 with moderate wildlife-livestock interactions and one in zone 3 with rare wildlife-livestock interactions. A total of 1,170 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). This study 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 seroprevalence of FMD 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 (high interface area) had a higher seroprevalence than zones 2 and 3 ($\chi^2 = 116.1$, $df = 2$, $p < 0.001$). 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 ($\chi^2 = 0.90$, $df = 2$, $p = 0.639$). Both serotypes A and O were more prevalent in zones 1 and 2 than zone 3 (low interface area) 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 with moderate and high wildlife-livestock interactions; 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 Maasai Mara National Reserve (MMNR).

Keywords: FMD, Seroprevalence, Risk factors, Serotype distribution, Land use change, Wildlife-livestock interface

5.2 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 buffaloes (*Syncerus caffer*) (Jamal & Belsham, 2013). The disease is a major challenge to livestock production in endemic areas as it causes significant production losses, including mortalities in calves, abortions, and reduced milk yields (Knight-Jones & Rushton, 2013). It is caused by the FMD virus (FMDV) of the genus *Aphthovirus*, within *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 parts of East Africa (Wekesa *et al.*, 2015). Serotype O accounts for majority of outbreaks in the region, with A, SAT 2 and SAT 1 following in decreasing importance (Wekesa *et al.*, 2013; Namatovu *et al.*, 2015). Multiple genetically distinguishable topotypes or strains may also occur within each serotype as FMDV has a high mutation rate (Brito *et al.*, 2017). Due to antigenic diversity between serotypes, recovery from one serotype does not provide cross-protection against other serotypes (Bari *et al.*, 2014).

The FMDV genome consists a single-stranded Ribonucleic Acid (ss RNA) of about 8400 nucleotides which encodes a polypeptide that is cleaved into several non-structural proteins (NSPs) and four structural proteins (SPs) (Jamal & Belsham, 2013). The 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, regardless of vaccination status, while the detection of anti-SPs among NSP positive animals is used to determine serotype responsible for the immune response (Namatovu *et al.*, 2015).

Knowledge on the epidemiology of FMD is limited in livestock and wildlife in the Maasai Mara ecosystem. The Maasai Mara ecosystem has diverse wildlife species which provide the locals with tourism-related revenues, in addition to livestock-derived livelihoods (Bedelian & Ogutu, 2017). Recent studies in the area have shown major land use changes that are attributed to increased human population, infrastructure development and land individualization (Løvschal *et al.*, 2017; Veldhuis *et al.*, 2019). 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 reserve, are examples of such land use changes (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 due to intensified interactions between wildlife and livestock (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, this study determined the seroprevalence of FMD in cattle across three zones with varying levels of wildlife-livestock interactions and identified putative risk factors associated with exposure. The study also determined FMDV serotypes circulating among cattle herds in the area. This study provides data on the current epidemiological situation of FMD in the area that can guide the identification of appropriate vaccines to use.

5.3 Materials and methods

5.3.1 Study site and sampling framework

The study area and compliance with ethical standards are described in **chapter 3** (section 3.3.1 and 3.3.2, respectively). The details of the sampled zones, selection of villages, study design, sample size estimation, collection of epidemiological data and blood samples are provided in chapter 4 (section 4.3). Besides the epidemiological data collected for brucellosis and leptospirosis (chapter 4), additional information on the putative risk factors of FMD was also recorded. For example, information was recorded on whether vaccination against FMD had been conducted in each sampled herd and whether sampled herds had experienced typical FMD signs such as blisters/lesions in the mouth, teat or hooves over the past year (appendix 3).

5.3.2 Serological testing

5.3.2.1 Detection of NSP antibodies against the FMD virus

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 done 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 done at ILRI, optical densities (ODs) of the samples and the 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;

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

Samples were classified as negative if the PI was < 50% and positive if \geq 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). 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;

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

Animals were classified as negative if the PP was < 10% and positive if PP was \geq 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).

5.3.2.2 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.

5.3.2.3 Interpretation of serology results

Animals were classified 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 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 indicative of animals having been vaccinated, while SP negatives corresponded to un-vaccinated and un-exposed individuals (Longjam *et al.*, 2011). In cases where sera from NSP positive animals showed seropositivity to more than one serotype, this study used differences in titres against serotypes of 3-4 folds to determine the serotypes responsible for the immune response. Animals were counted as having been exposed to infection with multiple serotypes when SP-antibody titres against two or more serotypes were not significantly different.

5.3.3 Data analysis

Results of serological analyses and the questionnaire data were entered into MS Excel (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 FMD virus 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 calculated 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 done using generalized linear mixed-effects models (GLMM). Data to these models were fitted 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. A saturated model with all significant predictors ($p < 0.05$) in the univariable models was first fitted and variables with $p < 0.05$ based on the Wald's χ^2 test retained. The final multivariable model was selected based on the lowest Akaike 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). The ICC for within-herd clustering of cattle was estimated using the *icc* function in *sjstats* package (Lüdtke, 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 having anti-SPs.

5.4 Results

5.4.1 Anti-NSP antibodies prevalence and distribution

A total of 1,170 cattle sera (78.6% and 21.4% females and males, respectively) from 390 herds were tested for antibodies against NSPs using two ELISA assays. The proportion of sampled herds indicated to have been vaccinated at the time of sampling was 44.9%. 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). The overall apparent animal-level and true seroprevalences of FMD were 83.8% (95% CI; 81.5–86.2) and 86.9% (95% CI; 84.5-89.1), respectively. The apparent animal-level seroprevalence of FMD differed significantly between locations where animals were kept. Zone 1 (high interface area) had a higher seroprevalence compared to zones 2 and 3 ($\chi^2 = 116.1$, $df = 2$, $p < 0.001$) (Table 5.1). The spatial distribution of NSP positive animals in the surveyed zones is shown in Figure 5.1. 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 5.1). The proportion of animals showing *Brucella* spp., *Leptospira* spp., (both presented in chapter 4) and FMD co-exposure was considerable (8.0%; 95% CI; 7.0-10.0), but not significantly different across zones.

Table 5.1: Variables associated with animal-level seroprevalence of FMD based on univariable logistic regression with random effect for herd

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				
Low interface area (zone 3)	240	63.3 (57.5 - 69.9)	1 (Ref.)	
Moderate interface area (zone 2)	465	83.4 (79.7 - 87.2)	3.6 (2.2-5.9)	< 0.001
High interface area (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.

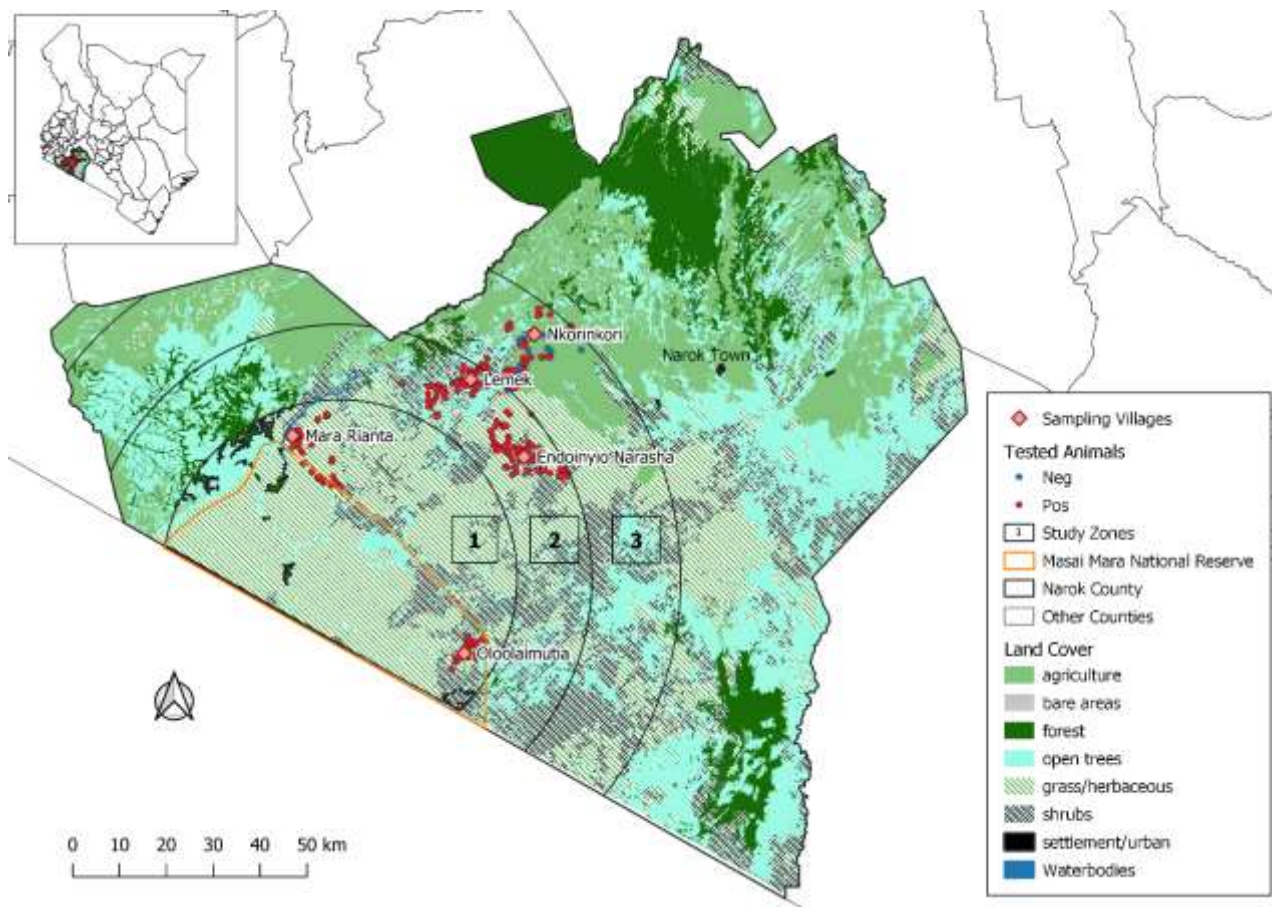


Figure 5.1: Map of Maasai Mara ecosystem showing the spatial distribution of NSP seropositive animals in the surveyed zones

5.4.2 Circulation of FMDV serotypes

Table 5.2 shows the results of the serotype-specific ELISA test (SPCE) and the distribution of circulating FMDV serotypes in the zones. A total of 49 (n = 932) 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 (low interface area) while serotype SAT 1, was higher in zone 3 compared to other zones. The estimated percentage of animals exposed to multiple serotypes was 18.0% (95% CI; 15.7-20.5). Across zones, there was a statistically significant difference in the proportion of animals showing serotype co-exposure ($\chi^2 = 8.16$, $df = 2$, $p = 0.017$).

Table 5.2: Distribution and seroprevalence of FMDV serotypes in various zones

FMDV serotypes	Zones							
	number of SP positive animals, percent (%) seroprevalence (95% CI)							
	Low interface area (zone 3)		Moderate interface area (zone 2)		High interface area (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.

5.4.3 Risk factors associated with animal-level FMD seropositivity

Table 5.1 shows important 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 zones with moderate and high wildlife-livestock interactions, mixing of cattle from multiple herds at watering points and pastoral husbandry practices as significant predictors of animal-level FMD seropositivity (Table 5.3). 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.

Table 5.3: Final multivariable model of animal-level risk factors for FMD in cattle based on GLMM analysis with a random effect for herd

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			
	Low interface area (zone 3)	1 (Ref.)	
	Moderate interface area (zone 2)	3.2 (1.9 – 5.5)	< 0.001
	High interface area (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).

5.5 Discussion

This study determined the current epidemiological status of FMD among cattle herds raised in three zones with different land use types and assumed to have varying levels of wildlife-livestock interactions in the Maasai Mara ecosystem. 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). This study

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 & 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 exposed animals become persistently-infected irrespective of their vaccination status (Barnett *et al.*, 2015). While the transmission risk of FMDV from carrier animals to naïve animals in herds is considerably low, but is still poorly understood (Arzt *et al.*, 2018), their existence in cattle populations can prevent farmers from accessing international markets for their animals or animal-source products due to the risk of introduction of the virus into FMD-free countries (Knight-Jones & Rushton, 2013).

The estimated ICC of 0.24 reported by this study was moderate, although studies elsewhere have reported high 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 considerably correlated. The disease is highly infectious and multiple animals within a herd often get 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 Maasai Mara area, female animals have lower offtake rates compared to males, as they are kept for milk production and breeding purposes by replacing animals lost during drought shocks (Huho *et al.*, 2011). The longer period over which females stay in herds compared to

males could lead to high chances of FMDV exposure or they may have repeated exposure to different FMDV serotypes or strains over time (Mesfinie *et al.*, 2019).

This study found that FMD seroprevalence increased as sites got closer to the MMNR. While this study did not quantify the level of interactions between livestock and wildlife in the targeted zones and thus this factor remains highly speculative, the close interactions between wildlife and livestock in zone 1 as animals are grazed in the MMNR and the surrounding wildlife conservancies, is a possible factor which could explain the higher seroprevalence of this disease in this zone compared to zones 2 and 3. Wildlife species are important sources of many pathogens shared with livestock (Bengis *et al.*, 2002). In the case of FMDV, the African buffalo (*Syncerus caffer*) is the primary reservoir host, especially for the SAT serotype. Other wildlife species may also act as temporary sources of infections when actively infected (Vosloo & Thomson, 2017b). While FMDV natural transmissions between buffalo and livestock have been shown in South Africa (Brito *et al.*, 2016) and Zimbabwe (Miguel *et al.*, 2013), the role of buffalo in the epidemiology of this virus in livestock populations in Eastern Africa is poorly understood (Teklehiorghis *et al.*, 2016).

This study showed that serotypes A, O, SAT 1 and SAT 2 were circulating in cattle, consistent with previous findings in the area (Bronsvort *et al.*, 2008; Wekesa *et al.*, 2015), but their relationships with those circulating in wildlife (e.g., buffaloes) were not determined since wildlife sampling was not undertaken. While SAT 1 and SAT 2 serotypes are presumed to be maintained by buffaloes, the seroprevalences of these serotypes were expected to be higher in zone 1 assumed to have higher levels of interactions between livestock and wildlife than zones 2 and 3. Nevertheless, the seroprevalence of SAT 1 was found to be higher in zone 3 compared to zones 1 and 2,

while that of SAT 2 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 shown genetically distinct SAT 1 and 2 isolates circulating in sympatric cattle and buffalo populations, an indication that wildlife species such as buffalo may have a limited role in the transmission of these serotypes to cattle, contrary to findings in South Africa (Brito *et al.*, 2016; Jori & Etter, 2016). It is therefore likely that the higher seroprevalence of SAT 1 in zone 3 compared to other zones could be due to livestock management factors rather than the interactions between livestock and wildlife. For instance, it is common for multiple herds within zone 3 to graze on post-harvest maize and wheat straws which may increase the transmission levels of this serotype via increased animal contact rates.

The different land use types adopted in the selected zones is also a possible factor which may explain the higher seroprevalence of FMD in areas close to MMNR (zone 1) than in zones 2 and 3. Land use change driven by the need to meet the demand for food, timber, fiber, water and other ecosystem services in many parts of the world (Mastel *et al.*, 2018) 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). Land use change, for example, can either inhibit or increase interactions between host species or affect their movements (Hassell *et al.*, 2017). These interactions (direct or indirect) may affect the level of microbe transmission between host species (Miguel *et al.*, 2013). For instance, in zone 1, cattle herds were grazed in MMNR and wildlife conservancies in predominantly pastoral systems. This type of livestock production involve herd movements in search of water and pasture, which may increase the chances of naïve cattle contacting 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 allow the utilization of grazing areas and watering sources by multiple herds which can lead to close interactions between animals, and/or contamination of these resources with animal fomites (Ayebazibwe *et al.*, 2010). Indeed, this study identified pastoral herd husbandry practice, sharing of grazing areas, and watering sources as significant predictors of FMD seropositivity in cattle.

This study also found that some of the NSP positive animals had serotype-reactive antibodies against more than one FMDV serotype. While it would be expected that pressure for co-exposure should be lower in zone 3, where NSP seroprevalence (virus circulation) is lower and distance from MMNR is increased, this study found significantly higher levels of co-exposure in zone 3 than other zones. This finding could be due to cross-reactivity between serotypes having common epitopes (Bari *et al.*, 2014), or an indication of 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 double or multiple serotypes exposure, as previous studies in East Africa and elsewhere, have shown that cattle are exposed to different serotypes with serotype predominance varying over time (Casey-Bryars *et al.*, 2018; Ouagal *et al.*, 2018).

5.6 Limitations

This study used 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 since animals were classified as either positive or negative based on a cut-off threshold value. Vaccinated-infected animals with little or no systemic

infections may also elicit non-detectable anti-NSPs immune response (Brocchi *et al.*, 2006). In contrast, animals vaccinated with non-purified vaccines may sero-convert 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 considerable proportion of the sampled herds were indicated to have been vaccinated during sampling (44.9%). 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 being affected by the different immune responses of individual animal tested and by the different possible immune statuses such as those that are vaccinated, infected, vaccinated and infected or vice versa, or those with multiple serotype infections. This study also used a cross-sectional study design and was not able to indicate which serotype causes frequent outbreaks in the area. While this study did not determine the level of livestock movements between the targeted zones at the time of sampling due to logistical constraints, a previous study conducted in the area indicated that livestock movements may be more prevalent during dry season than wet season, but is also depended on the cattle herd size (Butt, 2010). The selected zones were contiguous and thus this factor could have influenced the results of this study since it was not controlled for during the study implementation. Besides, the variable representing zones was entered as a fixed effect during the multivariable analysis rather than as a random variable.

5.7 Conclusions

This study showed higher FMD seroprevalence in areas close to the MMNR (zone 1) than zones 2 and 3 further away from MMNR with moderate and rare wildlife-livestock interactions respectively. The serotypes A, O, SAT 1 and SAT 2 were circulating in cattle with serotypes A and O being the most prevalent in zones with high and moderate interface than in that with low interface. SAT 1 was higher in the low interface zone than the other zones. The distribution of serotype SAT 2 did not vary significantly across zones. The vaccines used in the area should therefore include all the four serotypes, but also matched with circulating virus strains for improved efficacy. Vaccination interventions using multivalent vaccines can also be intensified in zone 1 with higher FMD exposure levels than zones 2 and 3. The establishment of FMD notification and surveillance system in the area is also required to ensure early case detection, timely response, and management of incidences as they occur. Future studies should also quantify how wildlife-livestock interactions and livestock movements may influence FMD incidence in the area for effective control of this disease. The sampling of wildlife species including buffaloes will also provide useful information on the genetic diversity of FMDV in the area.

CHAPTER SIX

6.0 MODELLING VACCINATION STRATEGIES AGAINST FOOT AND MOUTH DISEASE IN LIVESTOCK IN MAASAI MARA, KENYA

6.1 Abstract

Foot-and-mouth disease virus (FMDV) is a multi-host pathogen that affects cloven-hoofed animals, including livestock and wildlife. In pastoral herds, close interactions between cattle, sheep, goats and sometimes wildlife enhances FMDV transmission. More effective vaccination strategies are therefore required to better manage the disease in such circumstances. This study developed a Susceptible-Exposed-Infectious-Recovered (SEIR) model to predict the transmission dynamics of FMDV between cattle and sheep and analyzed the efficacy of alternative vaccination strategies to reduce transmission. Input parameters were obtained from literature and the vaccination scenarios tested included reactive vaccination of cattle alone, vaccination of both cattle and sheep hosts, and reactive and pre-emptive mass vaccination strategies. Relative efficacy of these approaches was assessed based on the cumulative incidence of FMDV in both cattle and sheep hosts. The effects of all vaccination scenarios were assessed by calculating the percentage (%) change in the cumulative incidence of FMD in cattle and sheep hosts relative to the baseline model with no intervention. The reactive vaccination of cattle alone with 100% coverage showed reduction in cumulative incidence of FMD in cattle and sheep populations by 4.23% and 0.04% respectively, while when both host species were vaccinated, the cumulative incidence reduced by 4.43% and 2.15% in cattle and sheep, respectively. The findings from this study also show that reactive vaccination can substantially reduce cumulative incidence of FMD if implemented immediately at the onset of the outbreak and with high coverage to compensate for the low vaccine efficacy. The cumulative incidence of FMD reduced by 3.18% and 1.61% in cattle and sheep populations, respectively, at day 1 of reactive mass vaccination with 75% coverage compared to 0.009% (cattle) and 0.005% (sheep) at day 15 with the same coverage. In pre-emptive vaccination strategy, the percentage of cumulative incidence of FMD averted increased as the time interval between vaccination and FMDV challenge increased. The findings of this study showed that both cattle and sheep hosts should be vaccinated in the Maasai Mara area for vaccination campaigns to have the desirable effect on FMD. Analysis from this model also indicate that both reactive and pre-emptive vaccination strategies can be used strategically to control FMD in the area. The model can also be used in subsequent studies for cost/benefit analyses of new vaccines such as the oil-based vaccine that is currently being piloted.

Keywords: FMD, cattle, sheep, incidence, vaccination strategies.

6.2 Introduction

Foot and mouth disease (FMD) caused by FMD virus (FMDV) is a highly contagious disease that affects domestic species such as cattle, pigs, sheep and goats (Arzt *et al.*, 2011), and multiple wildlife species including the African buffalo (*Syncerus caffer*) (Weaver *et al.*, 2013). As livestock production contributes significantly to the livelihood and food security of the Maasai pastoralists in Maasai Mara ecosystem (Nthiwa *et al.*, 2019), FMD outbreaks in the area can be social and economically devastating as the disease affects animals' welfare and productivity, and prevents farmers from accessing markets for their live animals or animal-source products such as milk and meat due to imposed trade restrictions (Knight-Jones & Rushton, 2013). Livestock production in the area is also carried out in wildlife-inhabited areas where interactions between wildlife and livestock occurs routinely at watering and grazing locations (Bedelian & Ogutu, 2017). Whereas studies in South Africa (Brito *et al.*, 2016) and Zimbabwe (Miguel *et al.*, 2017) have implicated wildlife species including buffalo as the main sources of FMDV in livestock, recent studies in the Maasai Mara ecosystem and in East Africa at large, have indicated that livestock-related factors such as increased stocking densities, sharing of watering and grazing areas, and livestock movements could be the major drivers of FMD outbreaks in livestock (Casey-Bryars *et al.*, 2018; Omondi *et al.*, 2019).

The Maasai Mara ecosystem has also experienced major ecological changes over the past decades, such as the decline of many wildlife species and rapid increase of cattle and small ruminants (goats and sheep) populations, especially in villages near the Maasai Mara National Reserve (MMNR) (Ogutu *et al.*, 2016; Løvschal *et al.*, 2019). For example, between 1977 and 2014, the populations of cattle and small ruminants

increased by 0.8% and 235.6%, respectively (Bedelian & Ogutu, 2017), while that of wildlife species decreased by 68% between 1977 and 2016 (Ogutu *et al.*, 2016). The main underlying factors for these changes include growth of human population and settlement expansion in areas adjacent to MMNR (Veldhuis *et al.*, 2019), land privatization and fencing (Løvschal *et al.*, 2017; Weldemichel & Lein, 2019), and climate change (e.g., frequent droughts) (Bartzke *et al.*, 2018) which have accelerated changes in land use strategies in the area for better utilization of the rangelands.

Despite small ruminants increasingly becoming important sources of household livelihoods in the Maasai Mara ecosystem due to their better drought tolerance (Løvschal *et al.*, 2019; Nthiwa *et al.*, 2019), the current vaccination efforts to control FMD by the National and County governments targets only cattle, yet infected small ruminants may act as sources of infections for cattle (Arzt *et al.*, 2011). The relative effectiveness of various vaccination strategies against FMD has also never been evaluated. This study developed a deterministic Susceptible-Exposed-Infectious-Recovered (SEIR) mathematical model to understand the theoretical transmission of FMDV between cattle and sheep using biological parameters of FMD obtained from scientific literature as input data. The baseline model framework was then used to assess the effects of vaccinating cattle alone versus vaccinating both cattle and sheep host species, and also to evaluate the effects of reactive and pre-emptive mass vaccination strategies. This model provides insights into the transmission dynamics of FMD in multi-species systems and can guide control policies for FMD in the area. The model could also be used for cost/benefit analyses of new vaccines such as the oil-based vaccine that is currently being introduced.

6.3 Materials and methods

6.3.1 Model formulation and description

Table 6.1 shows the biological parameters of FMDV obtained from published scientific literature used to simulate the theoretical transmission of FMDV between cattle and sheep populations in the Maasai Mara ecosystem. Cattle and sheep populations were grouped into four epidemiological classes (compartments) with respect to their FMD infection status. The compartments included the susceptible animals [S] (those non-infected, and that can become infected), the exposed animals [E] (those with latent infections – already infected but still unable to transmit the virus), the infectious animals [I] (those capable of transmitting FMDV to susceptible animals), and the recovered [R] (immune animals due to the effect of infection or vaccination – they are not infected or infectious, and cannot get infected because of their vaccine or infection-induced immunity) (Figure 6.1). The duration of immunity in the case of naturally-infected animals compared to vaccinated animals was assumed to be the same (Parida, 2009). The dynamic transitions of animals between the SEIR compartments were modelled using deterministic difference equations and discrete time intervals in days (appendix 4). To initiate FMD outbreak between cattle and sheep hosts, one infected animal for each host category was introduced in the model at a pre-determined day. The simulation of FMD outbreak with vaccination interventions being implemented at pre-determined days was simulated for 600 days. All simulations were done using R software, version 3.6.0 (R Core Team, 2019).

In the model, cattle and sheep populations were considered to be fixed (i.e., closed populations) thus the per capita birth rate of cattle (B_c) and sheep (B_s) were assumed to be equal to the respective cattle and sheep total mortalities (natural and FMD-

associated mortality) occurring at a rate of μ_c and μ_s in the various compartments of the respective host species. The model does not include intake and off-take of animals in herds. The ratio of cattle to sheep populations used in the model was 1:2 and was informed by a previous participatory study in the area which showed that sheep constitute about 40% of the herd composition compared to 28% for cattle (Nthiwa *et al.*, 2019).

While the transmission of FMDV occurs through various routes including aerosols (de Rueda *et al.*, 2015), this model assumed that transmission of the virus is primarily through animal contacts and that cattle and sheep hosts are well-mixed populations. In the cattle model, $\frac{1}{\delta_c}$ denotes the progression rate of individuals from exposed to infectious state, $\frac{1}{\gamma_c}$ is the average rate of infectiousness (i.e., mean recovery rate), while $\frac{1}{\alpha_c}$ is the rate at which recovered cattle lose immunity to become susceptible. In the case of sheep, the progression rate from exposed to infectious state, the mean recovery rate and the rate at which recovered sheep lose immunity to become susceptible were represented by $\frac{1}{\delta_s}$, $\frac{1}{\gamma_s}$ and $\frac{1}{\alpha_s}$, respectively. The parameters λ_c and λ_{sc} in the model represented the force of infection in cattle and sheep, respectively. The values used for these parameters are shown in Table 6.1. The flow rate of new infections in each host species per day was calculated by multiplying the total number of susceptible animals in each host category per day by the corresponding force of infection using the loop function in R software. This approach allowed the force of infection in susceptible animals in both host categories to scale linearly based on the number of infected animals per day (i.e., density-dependent transmission). The value of rate of transmission of FMDV (β), given as a product of the average number of contacts

between animals per day and the probability of transmission of the virus per contact (Hagenaars *et al.*, 2011) is shown in Table 6.1.

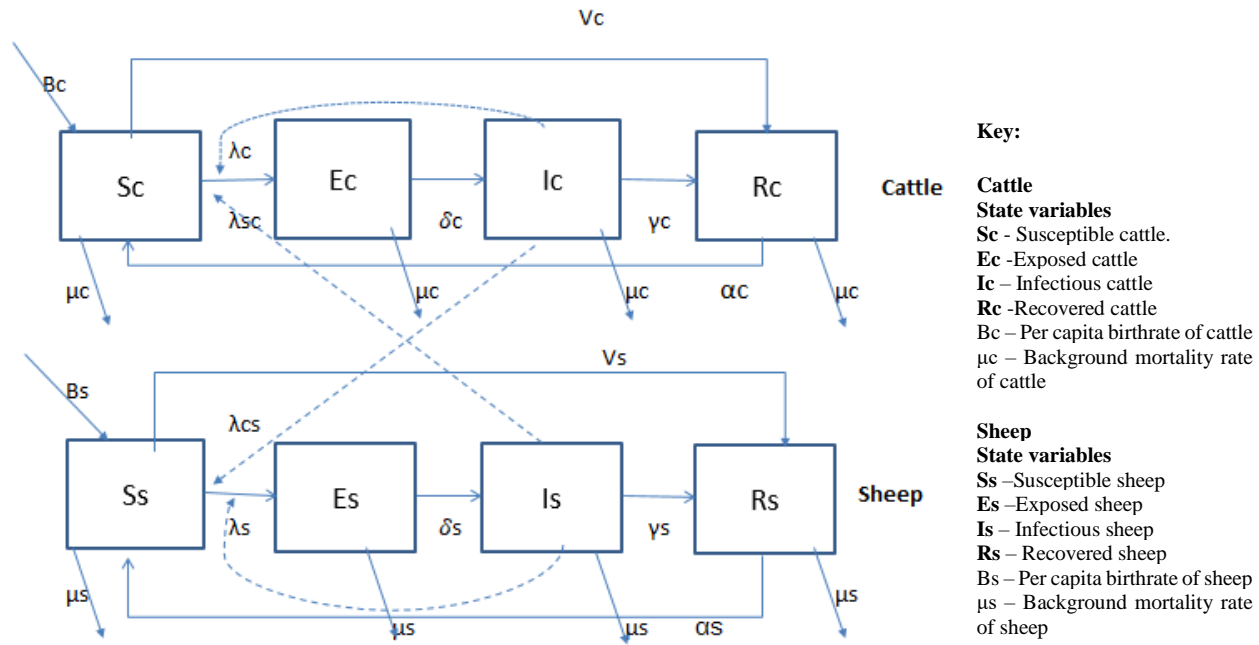


Figure 6.1: Model flow diagram showing the SEIR compartments in boxes and the transitions of animals between compartments by arrows.

Both V_c and V_s are additional transitions representing vaccination interventions implemented in cattle and sheep populations, respectively (Fig. 6.1). The dotted lines show compartments with influence on others.

Table 6.1: Literature biological parameters of FMDV used in the model

Parameters	Symbol	Values mean ^a (mn–mx)	Source
Cattle			
infectious period (days)	γ_c	8.5 (6.2-11.6)	(Yadav <i>et al.</i> , 2019)
exposed period (days)	δ_c	1.5 (1.1-2.1)	(Yadav <i>et al.</i> , 2019)
recovery period (days)	α_c	5 (4 - 6 months)	(Parida, 2009; Häsler <i>et al.</i> , 2017)
rate of transmission of fmdv of susceptible cattle by infectious cattle	β_c	0.008 ^b	estimate
force of infection of susceptible cattle by infectious cattle	λ_c	$_c_$	$(\beta_c \times ic)/nc[t]$ (computational parameter)
rate of transmission of fmdv of susceptible cattle by infectious sheep	β_{cs}	0.008 ^b	estimate
force of infection of susceptible cattle by infectious sheep	λ_{sc}	$_c_$	$(\beta_{cs} \times is)/ns[t]$ (computational parameter)
Sheep			
infectious period (days)	γ_s	3.3 (7-8)	(Alexandersen <i>et al.</i> , 2003; Mardones <i>et al.</i> , 2010)
exposed period (days)	δ_s	2 (2-7)	(Alexandersen <i>et al.</i> , 2003; Arzt <i>et al.</i> , 2011)
recovery period (days)	α_s	5 (4 - 6 months)	(Parida, 2009; Häsler <i>et al.</i> , 2017)
rate of transmission of fmdv of susceptible sheep by infectious sheep	β_s	0.008 ^b	estimate
force of infection of susceptible sheep by infectious sheep	λ_s	$_c_$	$(\beta_s \times is)/ns[t]$ (computational parameter)
rate of transmission of fmdv of susceptible sheep by infectious cattle	β_{sc}	0.008 ^b	estimate
force of infection of susceptible sheep by infectious cattle	λ_{cs}	$_c_$	$(\beta_{sc} \times ic)/nc[t]$ (computational parameter)

^a M, mean; mn, minimum; mx, maximum.

^b Estimate value used due to limited experiemntal data on this parameter.

^c The values for these parameters could not be indicated as they varied per day based on the number of infected animals.

$Nc[t]$, is the total population size of cattle per day $[t]$ given by the proportion of $Sc[t] + Ec[t] + Ic[t] + Rc[t]$.

$Ns[t]$, is the total population size of sheep per day $[t]$ given by the proportion of $Ss[t] + Es[t] + Is[t] + Rs[t]$.

6.3.2 Building of a baseline model

Single species infection models involving cattle or sheep populations alone (i.e., without inter-species interactions) were first fitted using the mean values of the published biological parameters of FMDV (Table 6.1). Thereafter, a baseline model with mixed populations of cattle and sheep and without vaccination interventions being implemented to either host was also fitted using the mean values of each parameter. Due to the large number of parameters used in the model, a series of sensitivity analyses were conducted to those with mean, minimum and maximum values to identify parameters which had significant effect on FMD incidence. The sensitivity analyses involved varying the values of each parameter one at a time from minimum to maximum and assessing the effects of the changes done by calculating the percentage (%) change in cumulative incidence of FMDV in cattle and sheep relative to the baseline model. As changes of values of the biological parameters of FMDV were expected to substantially affect the progression rate of FMD infections among animals, and ultimately the cumulative incidence of FMD (Yadav *et al.*, 2019), the baseline model described above simulated the average behavior of FMDV transmission between cattle and sheep populations in the area.

The basic reproductive ratio (R_0) defined as the average number of new infections arising from one infectious animal during the entire infectious period, in a population of susceptible animals (Heffernan *et al.*, 2005), was estimated using the single species infection models described above. Partial reproductive ratios (R_0^p) in cattle and sheep hosts were also estimated using the baseline model representing mixed-species infection systems. Both R_0 and R_0^p threshold values for each species were estimated from the peak of the outbreak using the formula; $\frac{1}{S[t]}$, where $S[t]$ is the critical

proportion of susceptible cattle or sheep at the time of the peak (Dohoo *et al.*, 2012). FMDV serotypes A, O, South African Territory [SAT] 1 and SAT 2 have been found to be circulating among livestock populations in the area (Wekesa *et al.*, 2015), but due to lack of serotype specific information on the parameters, this study assumed the exposed, infectious and recovery periods for all to be equal. Therefore, this study gives a global picture of FMD transmission in the area.

6.3.3 Vaccination scenario analyses

The effects of various vaccination strategies on the cumulative incidence of FMD in cattle and sheep hosts were evaluated using the baseline model framework. The FMD vaccines used in the analyses were considered to be multivalent and to confer cross-protection against multiple serotypes of FMDV circulating in the area (Häsler *et al.*, 2017). The vaccines were also estimated to have an efficacy of 85% in both host species (Parida, 2009). Vaccinated animals in both host species become protected against FMDV challenge within 7 days after vaccination (de los Santos *et al.*, 2018). The modelling of vaccination strategies proceeded by first calculating the proportion of susceptible cattle or sheep populations that should be vaccinated in single species and mixed-species infection systems to establish herd immunity (i.e., the critical vaccination coverage denoted as C_{vc}) using the corresponding basic reproductive ratio values for each host and the formula:

$$C_{vc} = \frac{1 - \frac{1}{R_0}}{h}$$

where h is the efficacy of FMD vaccine (Dohoo *et al.*, 2012). The effects of vaccinating cattle alone versus vaccinating both cattle and sheep were also assessed by implementing a vaccination coverage of 100% in cattle alone in the first scenario, and

to both host species in the second scenario on day 1 of the outbreak. This was followed by fitting a series of deterministic SEIR models to evaluate the effects of reactive and pre-emptive mass vaccination strategies on the cumulative incidence of FMD. The vaccination timings used for each vaccination strategy were purposefully determined. In the reactive vaccination strategy, animals were vaccinated on the 1st, 5th and 15th days after the outbreak while in pre-emptive vaccination strategy, they were vaccinated 5, 10 and 15 days before an outbreak. Varied vaccination coverages of 25%, 50% and 75% in both host species were assessed for each vaccination day. The effects of all vaccination strategies were evaluated by calculating the percentage (%) change in cumulative incidence of FMD in cattle and sheep hosts species relative to the baseline model.

6.4 Results

6.4.1 Cumulative incidence of FMD in cattle and sheep populations

The overall estimated cumulative incidences (i.e., the number of sick animals) of FMD in cattle and sheep host species were 1,460,078 (638,514 in the first year and 821,564 in the second year) and 1,126,807 (495,600 in the first year and 631,207 in the second year), respectively based on single species infection models. FMDV cumulative incidence increased by 6.9% and 11.4% in cattle and sheep hosts, respectively, in the mixed-species infection model. The baseline model predicted one major outbreak with its peak occurring on day 11 of the outbreak for both host species followed by a period of stable equilibrium (Figure 6.2). The estimated R_0 threshold values based on single species infection models were 24.4 and 3.0 for cattle and sheep, respectively. From the mixed-species infection model (baseline model), the R_0^p values were estimated to be 403.4 and 339.4 in cattle and sheep hosts, respectively.

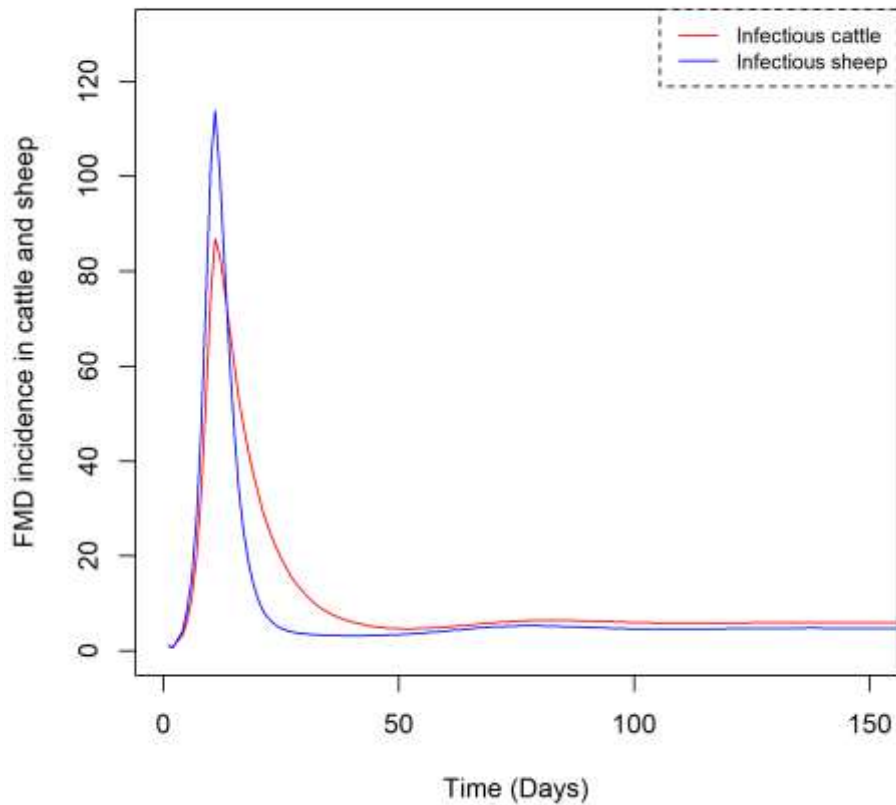


Figure 6.2: Predicted FMD incidence in cattle and sheep populations in the first 150 days of the outbreak.

6.4.2 Results of vaccination scenario analyses

The critical vaccination coverage calculated from the R_0 values of cattle and sheep were 96% and 67%, respectively, in single-species infection models. In mixed populations of cattle and sheep, the critical vaccination coverages were estimated at 100% for both species. The results obtained by reactive vaccination of cattle alone on day of 1 of the outbreak with 100% coverage showed reduction of cumulative incidence of FMD by 4.23% and 0.04% in cattle and sheep host species, respectively

(Figure 6.3). When both host species were targeted, the cumulative incidence of FMD dropped by 4.43% and 2.15% in cattle and sheep hosts, respectively (Figure 6.4).

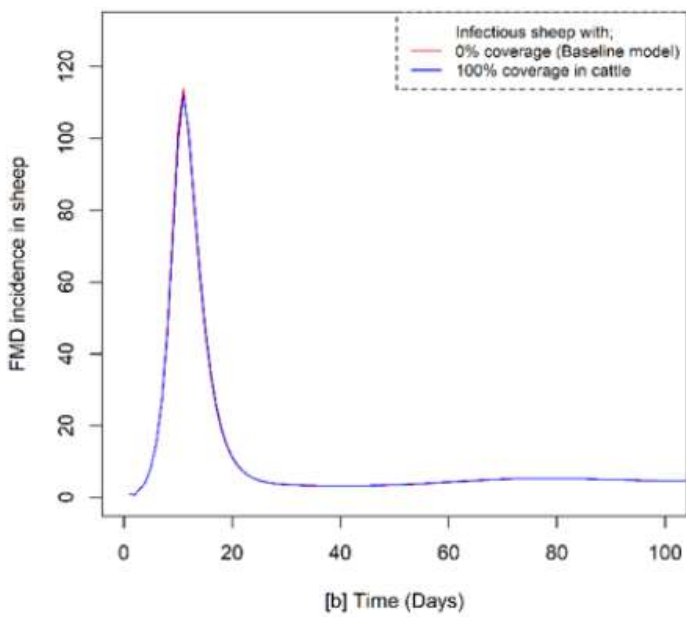
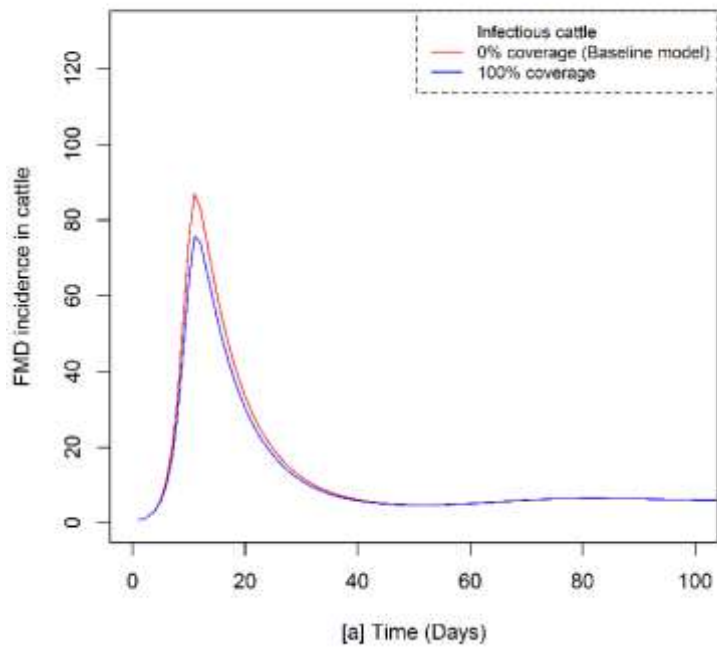


Figure 6.3: Estimated incidence of FMD averted in cattle [a] and sheep [b] by vaccinating cattle alone on day 1 of the outbreak compared to baseline model. Simulated incidence in the first 100 days of the outbreak.

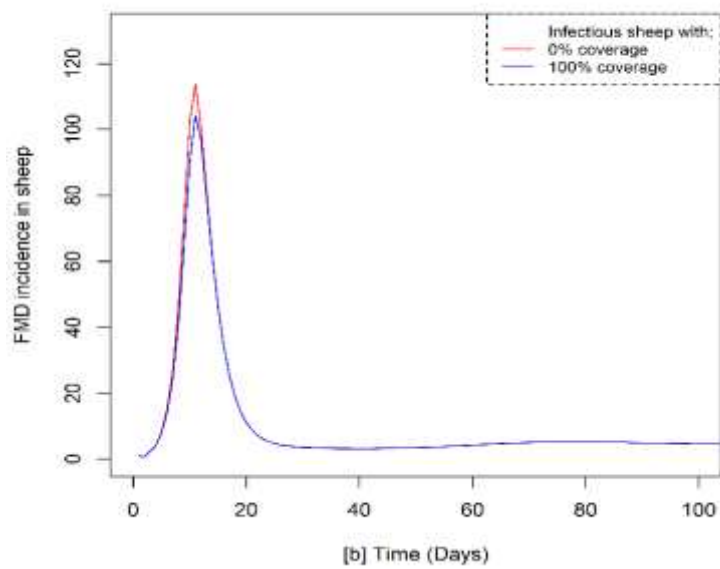
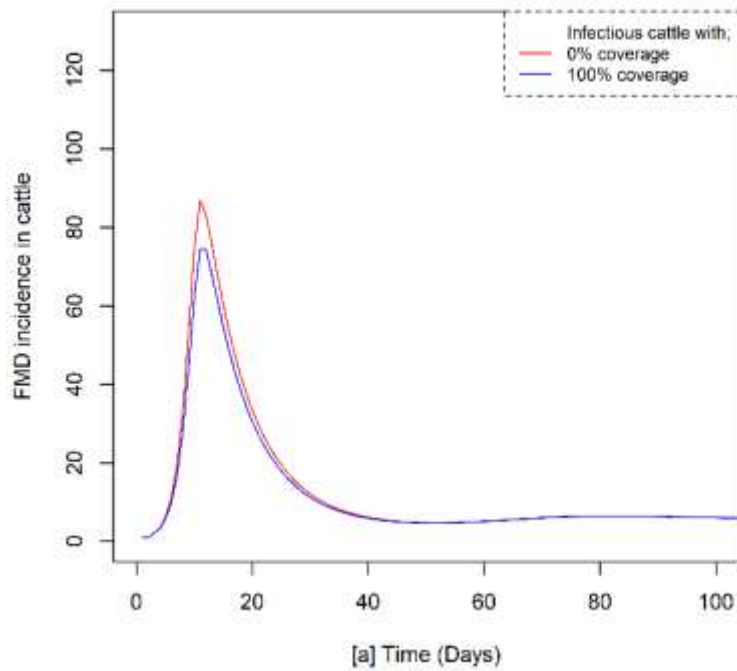


Figure 6.4: Estimated incidence of FMD averted in cattle [a] and sheep [b] by vaccinating both host species on day 1 of the outbreak compared to baseline model. Simulated incidence in the first 100 days of the outbreak

Table 6.2 shows the results of reactive and pre-emptive mass vaccination interventions implemented at different coverage levels. The results indicated that reactive mass vaccination can significantly reduce cumulative incidence of FMD if implemented immediately at the onset of the outbreak and with high coverage to compensate for the low vaccine efficacy. For instance, the cumulative incidence of FMD reduced by 3.18% and 1.61% in cattle and sheep, respectively, at day 1 of reactive mass vaccination with 75% coverage compared to 0.009% and 0.005% in cattle and sheep respectively, at day 15 with the same coverage. In the case of pre-emptive mass vaccination, the cumulative incidence of FMD decreased as the time interval between vaccination and FMDV challenge increased. In general, pre-emptive vaccination was shown to be more effective in reducing FMD cumulative incidence compared to reactive vaccination even when the former is implemented with low coverages (Table 6.2).

Table 6.2: Reduced cumulative incidence of FMD in cattle and sheep host species under different vaccination strategies and coverages

Vaccination strategy	Day implemented	Percentage (%) coverage	% cumulative incidence of FMD reduced in each host species	
			Cattle	Sheep
Reactive mass vaccination				
	Day 1 of the outbreak	25	1.06	0.54
		50	2.12	0.11
		75	3.18	1.61
	Day 5 of the outbreak	25	0.95	0.48
		50	2.00	0.96
		75	2.85	1.43
	Day 15 of the outbreak	25	0.003	0.002
		50	0.006	0.003
		75	0.009	0.005
Pre-emptive mass vaccination				
	5 days before the outbreak	25	75.54	10.25
		50	75.86	11.23
		75	76.16	12.20
	10 days before the outbreak	25	75.77	11.49
		50	76.10	12.42
		75	76.37	13.35
	15 days before the outbreak	25	76.00	12.71
		50	76.29	13.61
		75	76.57	14.50

6.5 Discussion

This is the first deterministic SEIR transmission model of FMDV developed between cattle and sheep host species in the Maasai Mara ecosystem. The higher cumulative incidence of FMD reported in mixed-species model than in single-species models involving either cattle or sheep populations could be associated with the high diversity of hosts in mixed-species systems which could increase the transmission of infectious pathogens including FMDV by creating a large pool of pathogen maintenance hosts

(Keesing *et al.*, 2010). The model predicted a higher cumulative incidence of FMD in cattle than sheep populations in the mixed species infection model. This finding could be due to differences in the transmission characteristics of FMDV between hosts. For example, the transmission of FMDV is estimated to be higher between cattle and cattle than from sheep to cattle (de Rueda *et al.*, 2014), while cattle are also more infectious compared to sheep (Mardones *et al.*, 2010; Backer *et al.*, 2012).

The estimated R_0 value in cattle (24.4) was quite high. However, these estimates were comparable to other studies e.g., 38.4 (range; 20-60) Haydon *et al.* (1997) and 2-70 (Woolhouse *et al.*, 1996). In the case of sheep, the estimated R_0 value (3.0) was also consistent with other studies, e.g., 1.1 (Orsel *et al.*, 2007b). The R_0^p values in cattle (403.4) and sheep (339.4) with inter-species transmission were extremely high. The R_0^p estimates are highly variable in different production systems which could be due to different levels of inter-species interactions. For instance, Orsel *et al.* (2007a) estimated R_0^p in cattle to be ∞ (1.3– ∞). The high R_0 and R_0^p values estimated by this study indicated that a high vaccination coverage would be required in both host species to establish herd immunity against FMDV. However, FMD is highly infectious and vaccination of cattle alone may be ineffective in reducing outbreaks if not implemented alongside other sanitary measures such as the restrictions of livestock movements to reduce exposure to FMDV and the isolation of infected animals (Woolhouse *et al.*, 1996). This observation was also reported by Knight-Jones *et al.* (2014) who demonstrated that vaccination against FMD alone can only suppress R_0 threshold value but not to below one.

The results also showed that substantial reduction of the cumulative incidence of FMD could be achieved if both cattle and sheep hosts are targeted during vaccination. Sheep,

goats and cattle are all susceptible to FMDV (Arzt *et al.*, 2011), and the presence of small ruminants in herds can modify the transmission dynamics of FMDV as they may act as maintenance hosts of the virus if not targeted during routine vaccinations or they may facilitate the mechanical transmission of FMDV through increased inter-species contacts (Barnett & Cox, 1999; de Rueda *et al.*, 2014).

This study found that reactive mass vaccination of both cattle and sheep hosts could significantly reduce cumulative incidence of FMD if implemented immediately at the onset of the outbreak and with high coverage to compensate for the low vaccine efficacy. Nevertheless, the implementation of this vaccination strategy may be faced with many logistical constraints as it assumes that FMD vaccines that match with the circulating FMDV serotypes/strains responsible for the outbreak will be produced on time. Furthermore, a well-equipped personnel (response team) will be required to achieve a high vaccination coverage which can lead to increased costs. For pre-emptive mass vaccination strategy, the cumulative incidence of FMD decreased as the time interval between vaccination and FMDV challenge increased. This finding is related to the time period the vaccine will take to become protective following vaccination. Pre-emptive vaccination strategy is more practical to implement in the area compared to reactive strategy as it can allow for pulse vaccination with low coverage to be conducted until the critical vaccination coverage necessary to establish herd immunity is achieved.

6.6 Limitations

The main limitation of this study is that the model used biological parameters of FMDV from literature instead of field incidence data to validate the model and that was due to cost constraints. The model does not account for herd dynamics due to

offtake of animals and/or purchase which could affect the transmission dynamics of FMDV if infected or susceptible animals were introduced in the model. The model is also non-spatial and does not account for varying livestock population densities within the area which can influence the transmission of FMDV especially in areas near MMNR with high livestock population densities. Besides, the model does not capture the seasonal (temporal) variations of FMDV transmission which is likely to increase during dry seasons when animals congregate at watering and grazing areas. Given the SEIR model framework used, animals were classified into four epidemiological classes and thus not allowing the accounting for individual variations in response to FMDV infections which can be done using agent-based models (Gilbert, 2008).

6.7 Conclusions

This study reported a higher incidence of FMD in mixed populations of cattle and sheep hosts than in single species infection models involving either host. The study also showed that both cattle and sheep hosts should be vaccinated in the area for vaccination campaigns to have the desirable effect on FMD. Pre-emptive vaccination strategy was also indicated to avert more cumulative incidence of FMD compared to reactive vaccination strategy at all the vaccination coverages considered. Further to this theoretical investigation, more studies are required to quantify the interactions between cattle and small ruminants and to determine the role of small ruminants in FMD epidemiology in the area.

CHAPTER SEVEN

7.0 CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

This study investigated how land use change, and hence the extent of livestock-wildlife interaction, influences the prevalence of infectious diseases in cattle in the Maasai Mara ecosystem. This study used FMD, *Brucella* spp. and *Leptospira* spp. as case study pathogens. This study also evaluated the effects of pre-emptive and reactive vaccination strategies in reducing the cumulative incidence of FMD in cattle and sheep. The findings from the focus group discussions showed that MCF, anthrax, FMD, CBPP, ECF and bovine trypanosomiasis were the most important diseases that had the highest economic impact on livestock production (chapter 3). Important zoonotic diseases including brucellosis and leptospirosis were not prioritized by pastoralist as important livestock diseases in the Maasai Mara ecosystem, although the laboratory testing of cattle for antibodies against these pathogens in the area showed high animal- and herd-level seroprevalences for both diseases (chapter 4). Nevertheless, the antibody testing used in this study indicated exposure and does not mean that animals had active or current infections. There is also limited data on the proportion of infected animals that develop clinical symptoms for both diseases. The lack of prioritization of brucellosis and leptospirosis could be also due to the low level of awareness of these diseases among communities living in the area. Both diseases are also neglected by the county and national governments in the area, and the vaccination of animals against them is rarely done which can partly explain the high exposure levels in animals (Njeru *et al.*, 2016). Besides, the main clinical manifestations of both diseases in animals include reduced milk production, abortions

and infertility (Adler & de la Peña Moctezuma, 2010; Franc *et al.*, 2018), which are non-specific as they are also associated with other diseases such as foot and mouth disease (FMD), contagious bovine pleuropneumonia (CBPP) and African animal trypanosomiasis (AAT), all prevalent in the area (chapter 3). The lack of distinct clinical syndromes for these pathogens indicates that syndromic diagnosis commonly used in participatory epidemiological investigations may be ineffective in discriminating between animals infected with *Brucella* spp. and *Leptospira* spp. pathogens from those infected with other diseases mentioned above. This finding also emphasizes the need to cross-check or triangulate syndromic diagnosis with laboratory testing of animals as a confirmatory test.

This study also found higher seroprevalences of FMD, *Brucella* spp. and *Leptospira* spp. among cattle raised in zones 1 and 2 (moderate to high interface areas) compared to zone 3 (chapters 4 and 5). Furthermore, the sampled animals had a considerable level of co-exposure for the targeted pathogens although this estimate was did not differ significantly between zones. This finding indicated that animals in Maasai Mara ecosystem may be exposed to multiple pathogens, either concurrently or in sequence during their lifetime. The exposure of animals to multiple pathogens could be due to shared risk factors for these pathogens. For example, the sharing of grazing areas and watering locations were identified as important predictors for the seropositivity of these pathogens. The sharing of these resources may increase the contacts of animals between and within herds which could influence the transmission levels of these pathogens. The concurrent exposure of animals to multiple pathogens in the area can result in different health outcomes in the animal as pathogens may interact directly or indirectly due to shared environment (i.e., host) and resources (Rynkiewicz *et al.*,

2015). Multiple pathogens exposure in animals, for example, may increase their susceptibility to secondary infections or lead to increased competition for infections sites between pathogens and energy between pathogens and host cells. Ultimately, this can significantly affect the health of the animal including its productivity and survival (Smith & Holt, 1996). This finding suggests the need to consider co-infections in epidemiological investigations rather than focusing on a single pathogen as animals may be co-infected with multiple pathogens in their natural environments.

Although information on the role played by small ruminants in the epidemiology of the target pathogens is very limited in the area, the transmission model built for FMD indicated that the inter-species interactions between cattle and sheep in the area can significantly increase the cumulative incidence of this disease in both hosts (chapter 6). The results of pre-emptive and reactive vaccination interventions indicated that both can be used strategically to control FMD in cattle and small ruminants in the area. Transmission models were not developed for *Brucella* spp. and *Leptospira* spp. due to limited data on the biology and ecology of these pathogens in the area.

7.2 Recommendations

This study provides current data on how varied land use types and different levels of wildlife-livestock interactions may influence the seroprevalence of FMD, *Brucella* spp. and *Leptospira* spp. in the Maasai Mara ecosystem. The targeted pathogens were found to have a considerable level of co-exposure and thus there is need to develop an integrated control strategy for these pathogens in the area.

1. The vaccination of cattle against *Brucella* spp. and *Leptospira* spp. which is rarely done in the area should be considered and can be implemented together with that of FMD provided that interferences between the vaccines being used

does not occur and that cost-effectiveness studies confirmed the viability of vaccination.

2. An active surveillance and monitoring system for the targeted pathogens should also be established to enhance timely diagnosis and control of these diseases.
3. The effects of co-infections or exposure of the targeted pathogens in cattle should also be quantified in the area as this could guide the devise of more effective control interventions.
4. While the level of awareness of brucellosis and leptospirosis as zoonotic diseases is low in the area, there is need to increase community awareness on the transmission pathways of these diseases, their prevention and control. This information can be provided in the form of crafted messages that can be disseminated using the local stations, village meetings, churches and schools to have a wider coverage.

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APPENDICES

Appendix 1: ILRI Institutional Research Ethical approval



29 September 2016

Our Ref: ILRI-IREC2016-02

International Livestock Research Institute
P.O. Box 30709 00100
Nairobi, Kenya.

Dear Prof. Eivin Røskoft,

RE: LINKING BIODIVERSITY, ECOSYSTEM FUNCTIONS AND SERVICES IN THE SERENGETI-MARA REGION, EAST AFRICA: DRIVERS OF CHANGE, CAUSALITIES AND SUSTAINABLE MANAGEMENT

Thank you for submitting your request for ethical approval to the ILRI Institutional Research Ethics Committee (ILRI IREC). ILRI IREC is accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya.

This is to inform you that ILRI IREC has reviewed and approved your study titled '**Linking Biodiversity, Ecosystem Functions and Services in the Serengeti-Mara Region, East Africa: Drivers of Change, Causalities and Sustainable Management**'. The approval is for the epidemiological components only and does not include socio-economic aspects (covered under Work Package 4 Task 4.2). The approval period is September 29, 2016 to September 28, 2017 and is subject to compliance to the following requirements:

- Only approved documents will be used;
- All changes must be submitted for review and approval before implementation;
- Adverse events must be reported to ILRI IREC immediately;
- Submission of a request for renewal of approval at least 30 days prior to expiry of approval period; and
- Submission of an executive summary report within 90 days upon completion of the study.

This approval is given for the epidemiological components only and does not include socio-economic aspects (covered under Work Package 4 Task 4.2).

Please do not hesitate to contact ILRI IREC on ILRIResearchcompliance@cgiar.org for any clarification or query.

Yours Sincerely,

**Dr. Silvia Alonso (pp. Jane Poole, Statistician, ILRI IREC)
Chair, ILRI Institutional Research Ethics Committee**

Documents received & reviewed:

- Research compliance form and IREC form 2
- PEN Technical Guidelines v4
- PEN Prototype Questionnaire v4.4

Appendix 2: ILRI Institutional Animal Care and Use Committee approval



9th December 2019

Our Ref: ILRI-IACUC2016-20

International Livestock Research Institute
P.O. Box 30709 00100
Nairobi, Kenya.

Dear Bernard Bett, PhD & Daniel Mutiso,

Ref: Foot and Mouth Disease (FMD) cross sectional study and outbreak investigation in Maasai mara, Narok

This is to inform you that ILRI IACUC has reviewed and approved your request to use animals in your research activity titled '*Foot and Mouth Disease (FMD) cross sectional study and outbreak investigation in Maasai mara, Narok*' as per the IACUC - ANIMAL USE FORM approved on 3rd October 2016. The approval is subject to compliance to the following:

- Compliance to applicable regulatory requirements and submission of documentary evidence;
- Minor changes required must be submitted to ILRI-IACUC for review and approval using the IACUC - ANIMAL USE MINOR AMENDMENT FORM before implementation.
- Only persons named on the approved IACUC - ANIMAL USE FORM (section 3 - 5) shall be allowed to handle and/or carry out sampling of the animals during this activity.
- Submission of completed PI Report Back Form upon completion of this activity to the ILRI IACUC.
- Reporting of any adverse events to ILRI IACUC immediately.

Please do not hesitate to contact ILRI IACUC on ILRIResearchcompliance@cgiar.org for any clarification or query.

Patron: Professor Peter C Doherty AC, FAA, FRS

Animal scientist, Nobel Prize Laureate for Physiology or Medicine–1996

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ILRI has offices in East Africa • South Asia • Southeast and East Asia • Southern Africa • West Africa

Yours Sincerely,



**Dr Roger Pelle (pp. Jane Poole, IACUC Biometrician)
Chair, ILRI Institutional Animal Care & Use Committee**

Documents received & reviewed:

- IACUC - Animal Use Form
- revisions of IACUC form during review process

Appendix 3: Questionnaire

Index case details for each epidemiological unit		
Date of form entry:	Farm owner: Years of operation	Farm name:
Farmer's Telephone Number: (optional)	Cattle herd size: Proportions of adults/weaners >1 years..... Calves <1 years) Goats Nos..... Sheep Nos.....	Herd vaccination status/history of the herd. (Dates since the last vaccination and vaccines administered)
Location of the farm: (Village, Sub-location and Location)	GPS coordinates of the farm: Herd management practice (pastoral or sedentary system)	
Type of sample collected: a) Blood..... b) Oral pharyngeal / throat or buccal cavity swabs or epithelium samples/lesions c) Milk.....		
Sample reference number (should match the ear tag on the sampled animal)		
The cattle breed sampled: a) Local breed eg Zebu or Borana b) Others (specify)..... ... c) Sex of the sampled cattle..... d) Age (approximate).....		

Exposure factors' trace back: Farmers' interviews

1. Which areas did you graze?
2. Do you have communal grazing areas/ grazing reserves within the village?
 - a. Yes b. No
3. Do farmers within the village share these grazing areas?
 - a. Yes b. No
4. Do farmers from other villages share the communal grazing areas?
 - a. Yes b. No

If yes, when?

5. Do your cattle graze in the national park?
 - a. Yes b. No
6. Does your livestock mix with other herds in the grazing areas?
 - a. Yes b. No
7. How many herds do they mix with while grazing?
 - a. Daily: _____ b. Weekly: _____ c. Monthly: _____
8. Did any of the herds your livestock mixed with while grazing have FMD in the last month?
 - a. Yes b. No c. Unsure
9. Do you sight wildlife near your livestock at grazing or when on transhumance?
 - a. Yes b. No
10. Which types of wildlife do you see near your livestock?
 - a. Ungulates b. Predators c. Monkeys d. Other.
11. How do you experience the contact with wildlife?

- a. Positive b. Negative c. Indifferent.

12. Which species of livestock do you see?

- a. Goats b. Sheep c. Pigs d. Poultry e. Other

13. Do you have a water source for your herd within the farm?

- a. Yes b. No

14. Are these water sources shared with other herds within the village?

- a. Yes b. No

15. Do neighboring villages share these watering points?

- a. Yes b. No

16. How many other herds use this watering point for their livestock?

- a. 1-5 b. 5-10 c. 10-15 d. 15-20 e. >20

- f. None

17. Do your cattle mix with other herds at watering point? How many herds do they mix with when they drink?

- a. Yes b. No

If yes, how many?

18. Does your livestock share trek routes with herds?

- a. Yes b. No c. Unsure

19. Do your herd share water points on the trek with other herds?

- a. Yes b. No c. Unsure

20. Did the neighboring farmers have FMD in the last one year?

- a. Yes b. No c. Unsure

21. Are there herds within the village that you know have FMD?

- a. Yes b. No

22. Did you buy any livestock in the last one year? How many, species and which market?

- a. Yes b. No

If yes, please specify:

23. Type of husbandry eg breeding bull, AI, breeding bull own, common use, breeding bull from another farm etc

Please specify:

24. Are there dipping points common for the locals? {as areas where cattle mix freely hence are foci of FMD, brucellosis and leptospirosis

- a. Yes b. No

25. Do the personnel working on the premises visit other livestock holdings, for instance the veterinary officers, milk collectors and artificial inseminators?

- a. Yes b. No

26. Do vehicles have free access to the farm?

- a. Yes b. No

27. Have you noticed any of the following signs of illness in your cattle?
(Choose as many as needed):

- a. Fatigue
- b. Loss of pregnancy/abortion/stillbirth
- c. Decrease in milk production
- d. Mastitis/udder swelling and/or pain
- e. Unwillingness to walk/stand
- f. Fever
- g. Blisters in mouth, teats or hooves?

28. Have any of the people handling the animals experienced:

- a. Fever
- b. Sweat
- c. Malaise
- d. Headache
- e. Pain in muscles, joints and/or back (Choose as many as needed)

Interviewer's Details

Name.....Signature.....Date.....

Appendix: 4: Difference equations used to model the transmission of FMD virus between cattle and sheep

Days = 600

Cattle parameters

Sc = rep(120,days) # initial number of susceptible cattle
Ec = rep(0,days) # Initial number of exposed cattle
Ic = rep(1,days) # Initial number of infected cattle
Rc = rep(0, days) # Initial number of recovered cattle
bc = 0.008 # rate of transmission of susceptible cattle by an infectious cattle
ac = 1/150 # rate of recovery of infected cattle
dc = 1/1.5 # progression rate of exposed cattle into infected compartment
gc = 1/8.5 # mean recovery rate of infected cattle
cum_inc = rep(1,days) # cumulative count of infected cattle

Sheep parameters

bs= 0.008 # rate of transmission of susceptible sheep by an infectious sheep
as=1/150 # rate of recovery of infected sheep
gs=1/3.3 # an recovery rate of infected sheep
ds=1/2 # progression rate of exposed sheep into infected compartment
Ss=rep(240,days) # initial number of susceptible sheep
Es=rep(0,days) # initial number of exposed sheep
Is=rep(1,days) # initial number of infected sheep
Rs=rep(0, days) # initial number of recovered sheep
Cum_cases=rep(1,days) # cumulative count of infected sheep

Model equations used to model transmission of FMD virus among cattle and sheep single species populations

```
for(i in 1:days){  
  Sc[i+1]=Sc[i]-(Sc[i]*bc*Ic[i])+(Rc[i]*ac)  
  Ec[i+1]=Ec[i]+(Sc[i]*bc*Ic[i])-(Ec[i]*dc)  
  Ic[i+1]=Ic[i]+(Ec[i]*dc)-(Ic[i]*gc)  
  Rc[i+1]=Rc[i]+(Ic[i]*gc)-(Rc[i]*ac)  
  cum_inc[i+1]=(cum_inc[i])+Ic[i+1]  
  Ss[i+1]=Ss[i]-(Ss[i]*bs*Is[i])+(Rs[i]*as)  
  Es[i+1]=Es[i]+(Ss[i]*bs*Is[i])-(Es[i]*ds)  
  Is[i+1]=Is[i]+(Es[i]*ds)-(Is[i]*gs)  
  Rs[i+1]=Rs[i]+(Is[i]*gs)-(Rs[i]*as)  
  Cum_cases[i+1]=(Cum_cases[i])+Is[i+1]}
```

Equations used to model the transmission of FMD virus between cattle and sheep mixed-species populations

cat_SR=0.008 # rate of transmission of FMD virus of susceptible sheep by infectious cattle

SR_cat=0.008 008 # rate of transmission of FMD virus of susceptible cattle by
infectious sheep

ncat= Sc+Ec+Ic+Rc # number of cattle at any day during the simulated epidemic

```
for(i in 1:days){  
  Sc[i+1]=Sc[i]-(Sc[i]*bc*Ic[i])-(Sc[i]*SR_cat*Is[i])+(Rc[i]*ac)  
  Ec[i+1]=Ec[i]+(Sc[i]*bc*Ic[i])+(Sc[i]*SR_cat*Is[i])-(Ec[i]*dc)  
  Ic[i+1]=Ic[i]+(Ec[i]*dc)-(Ic[i]*gc)  
  Rc[i+1]=Rc[i]+(Ic[i]*gc)-(Rc[i]*ac)  
  cum_inc[i+1]=(cum_inc[i])+Ic[i+1]  
  ncat[i]<-Sc[i]+Ec[i]+Ic[i]+Rc[i]  
  Ss[i+1]=Ss[i]-(Ss[i]*bs*Is[i])-(Ss[i]*cat_SR*Ic[i])+(Rs[i]*as)  
  Es[i+1]=Es[i]+(Ss[i]*bs*Is[i])+(Ss[i]*cat_SR*Ic[i])-(Es[i]*ds)  
  Is[i+1]=Is[i]+(Es[i]*ds)-(Is[i]*gs)  
  Rs[i+1]=Rs[i]+(Is[i]*gs)-(Rs[i]*as)  
  Cum_cases[i+1]=(Cum_cases[i])+Is[i+1]}
```