SERO-PREVALENCE AND RISK FACTORS ASSOCIATED WITH *Coxiella burnetii* FROM CATTLE IN THE MASAAI MARA ECOSYSTEM IN NAROK, KENYA

DR. GRACE ANGELINE WANJIKU WATENE

(BVM- UNIVERSITY OF NAIROBI)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR MASTER OF VETERINARY PUBLIC HEALTH

FACULTY OF VETERINARY MEDICINE UNIVERSITY OF NAIROBI

2021

DECLARATION

This is my original work and has not been submitted to any institution for academic purposes.

Dr. Grace Angeline Wanjiku Watene Signature……Date 29th November 2021

Declaration by Supervisors:

This thesis has been approved with our approval as the university of Nairobi supervisors

Dr. Gabriel Aboge, (BVM, MSc & PhD)

Department of Public Health, Pharmacology and Toxicology, University of Nairobi

Signature……Date… 29th November 2021 ……

Prof. George K. Gitau (BVM, MSc & PhD) Department of Clinical Studies, University of Nairobi

Signature Date 29th November 2021

DEDICATION

I dedicate this work to my parents whose prayers have been a solid rock and my brothers, a source of continuous encouragement. This work is also dedicated to my best friends Dr. Hellen Akoth and Dr. Tequiero Abuom.

ACKNOWLEDGMENTS

Many thanks to the University of Nairobi and my supervisors; Dr. Gabriel Aboge and Prof. George Gitau who mentored me, directed me into the right focus and supported me throughout my work. This study was made possible through the support of Dr. Bernard Bett (ILRI) who accorded me the study samples and laboratory support. The laboratory assistance of Richard Nyamota and James Akoko was well received as they guided me through the laboratory protocols and testing. Their willingness to provide feedback made the successful completion of this research study. A special thanks to Dr. Daniel Nthiwa (ILRI); who generously aided me with his previous study data and helped with the statistical analysis. I also wish to appreciate Dr. Tequiero Okumu Abuom and Dr. Hellen Akoth who have been a source of encouragement and academic friends throughout my MSc studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

^{0}C	Degrees Centigrade
CI	Confidence Interval
CFT	Complement Fixation Test
C. *burnetii*	*Coxiella burnetii*
ELISA	Enzyme Linked Immunosorbent Assay
IFAT	Indirect Fluorescent Agglutination Test
ILRI	International Livestock Research Institute
min	Minute(s)
ml	Milliliter
NCIA	Negative control 1 in sample A
NC2A	Negative control 2 in sample A
NCX	Negative control average
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PCX	Positive control average
s	Seconds
Spp	Species
µl	Micro-liter
WHO	World Health Organization

ABSTRACT

In Sub-Saharan Africa, livestock keeping is the backbone of livelihoods for most inhabitants and plays a key role in rural poverty alleviation strategies. In countries like Kenya, livestock keeping has a higher socio-economic role; people's livelihoods are intertwined with those of their livestock with a bigger proportion of people directly dependent on livestock products and indirectly through the income generated through livestock sales and their products or employment in livestock-related sectors. Infectious diseases such as Q fever caused by *Coxiella burnetii* in the livestock industry affects its productivity and ability to support livelihoods. The aim of this study was to conduct a seroprevalence and risk factor analysis of *C. burnetii* in cattle in Mara ecosystem in an effort to strengthen baseline data.

589 samples were collected in a cross sectional design-multistage sampling and stored at a Sera bank at the International Livestock Research Institute (ILRI). These sera samples were then tested for the Q fever antibodies using IDEXX ELISA. The sera were previously collected from 390 herds in 5 different villages from 3 zones subdivided based on the livestock-wildlife interactions and land use patterns. Zone was 1 characterized by extensive pastoralism where animals grazed in surrounding wildlife reserves, zone 2 was characterized by sedentary husbandry system with animal grazing zones fenced and zone 3 was characterized with mixed agriculture and livestock systems.A questionnaire was used to collect data on farm and animal levels factors.

The sera samples were analyzed using an IDEXX ELISA kit to detect the presence of *Coxiella burnetii* IgG antibodies which were measured by means of colorimetry. Univariable and multivariable logistic regression analyses were used to analyze for the risk factors for *C. burnetii* seropositivity, with the outcome variable being the exposure to *C. burnetii* antibodies

The seroprevalence of *C. burnetii* was (1.9%) where 11 samples revealed the presence of C.

burnetii IgG antibodies. Zone 3 with a lower livestock-wildlife interaction, had a higher seroprevalence [2.48% (CI 0.51 ± 7.07)] than zone 1, which had higher interaction at the livestock-wildlife interphase [1.75% (CI 0.48 ± 4.42)]. Significant risk factors on univariate analysis at animal level was bull ownership {OR=4.8 (CI 0.4±16.2) P value 0.016}, while at at the herd level the significant variables were animals grazing in shared areas between villages {OR 2.3CI 1.0 ± 5.0) P value 0.046}, mixing of cattle in different herds had an {OR 0.4 CI 0.2 ± 0.9 P value 0.029}, herds that had newly purchased livestock {OR 10.1 CI 1.3±75.8 P value 0.025}, bull ownership P value 0.003, history of abortion in the herd { OR 0.3 CI 0.1 ± 10.9 P value 0.018} and communal grazing had a borderline significance of (OR=2.2 P-Value). At the multivariable analysis bull ownership was the only significant variable {OR 5.2 CI 1.5 ±18.4) P value 0.010).

The study confirmed the previous exposure of the cattle in Mara ecosystem to the *Coxiella burnetii* representing the risk to human transmission and qualifying the need to have better veterinary and public health interventions.

CHAPTER ONE: INTRODUCTION

1.0 Background information

In Sub-Saharan Africa, livestock keeping is the backbone of the livelihoods for most inhabitants and plays a key role in rural poverty alleviation strategies (Thornton, 2010). In countries like Kenya, livestock keeping has a significant socio-economic role; people's lives are intertwined with those of their livestock with a bigger proportion of people directly dependent on livestock products and indirectly through the income generated through livestock sales and their products or employment in livestock-related sectors (Thornton, 2010; IGAD, 2011; Bettencourt et al., 2015). The livestock industry however suffers a lot of challenges such as diseases and climatic changes that affect its productivity and ability to support livelihoods. Amongst the diseases, are notifiable infections such as brucellosis, rift valley fever and Q fever (Query fever) that not only occur in animals, but also spreads to man and can cause serious outcomes.

Q fever has been categorized internationally as one among the top thirteen global significant zoonoses, and has been labeled among the most contagious infectious disease (Grace et al., 2012; Njeru et al., 2016a; Danka et al., 2019; Johnson et al., 2019a). The disease is mainly characterized by abortion in domestic food animals and, acute to chronic disease in humans (Njeru et al., 2016a; Johnson et al., 2019b). Cattle, sheep and goat act as the key reservoirs of the infection/disease with transmission to people predominantly achieved through inhalation of dust particles laden with the bacteria antigen (Angelakis and Raoult, 2010a). *C. burnetii* can also cause disease in other species including domestic dogs and cats, reptiles, arthropods and birds (Johnson et al., 2019a).

Q fever outbreaks in human and domestic animals have been documented in many developed countries (To et al., 1996a; Bernit et al., 2002a; Gidding et al., 2009a; Delsing et al., 2010a; de Lange et al., 2015; Koka et al., 2018). In 2007 -2009 the largest-ever recorded outbreak of

Q fever involving over 3,500 human cases occurred in the Netherlands highlighting the public health impact of the disease (Delsing et al., 2010b).The outbreak first emerged in two dairy goat farms in the South of the country then later human beings were affected with 20% being hospitalization cases. The priority risk factor during that outbreak was the radial distance proximity of the human beings and these two dairy goat farms (van der Hoek et al., 2012).The disease is most likely underdiagnosed in Africa since it is not routinely tested in suspected species (Vanderburg et al., 2014a; Mwololo et al., 2014a; Njeru et al., 2016a; Eldin et al., 2017). In Kenya, only two outbreaks have been detected in human beings in 2000 and 2014 involving 81 people in the Rift Valley Region. Many other cases of Q fever have been documented incidentally as cases of undiagnosed fevers and pneumonias with high exposure rates being detected in humans and animals (Potasman et al., 2000a; Zoonotic Disease Unit, 2014a; Knobel et al., 2013a; Njeru et al., 2016a).

There was study by (Nguro, 2017) done to investigate the influence of wildlife on livestock *C. burnetii* infections. A seroprevalence of 25% was observed in Impalas (A*epyceros melampus*). Although the study sample size was relatively low, this showed that wildlife-livestock interactions have led to increased disease on both interfaces. In the same study the seroproportion of sheep was 6% and in goats 21.7%.

This study investigated the seroprevalence of coxiellosis in Mara Region Narok County, an intricate ecosystem where livestock and human beings interact. This interaction may result in disease transmission across wildlife- livestock and humans.

1.1 Problem Statement

Q-fever is characterized by variable range of clinical presentation in both animals and human beings. The infection can also be asymptomatic in animals with the diagnosis of the infection mainly relying on the reproductive failures characterized by birth of weak off springs, late gestation abortions and acquired infertilities (Psaroulaki et al., 2006). Since the general

reproductive symptoms can also be observed in other diseases, there is a risk of misdiagnosis and a true diagnosis can only be confirmed by laboratory analysis. Subsequently, serological tests such as ELISA, have been reliably used for routine screening of *C. burnetii* antibodies with final confirmation of clinical infection achieved by molecular techniques. Q fever impacts livelihoods of livestock farmers through losses in reproduction. The disease is also of public health importance being a zoonotic disease. Outbreaks of Q fever in both human and domestic animals have been reported in many developed countries. In Africa, the disease is most likely underdiagnosed since it is not routinely tested in susceptible species. Subsequently, since Q fever has been generally neglected in Kenya , the epidemiology of the disease is still not clearly understood both in the animal and human health systems because of limited availability of technical and financial capacity to allow for significant epidemiological studies

1.2 Objectives of the study

1.2.1 General objective

The general objective of the study was to determine the sero-prevalence and risk factors associated with *C. burnetii* in cattle in Mara region of Narok County, Kenya

1.2.2 Specific objectives

To determine the seroprevalence of *C. burnetii* in cattle in the Mara region; of Narok County, Kenya.

To determine the risk factors associated with *C. burnetii* seroprevalence in cattle in Mara region of Narok County, Kenya.

1.3 Justification

There is extensive pastoralism in Kenya and large heads of livestock and wildlife interact freely at the human-livestock-wildlife interface. The latter serves as an ideal environment for Q fever cross-infection and enables researchers to study the transmission dynamics of this zoonotic disease. There is an overwhelming indication for current research to put focus on Q fever so that its epidemiology can be better understood for better surveillance and effective control. This study will provide relevant data for veterinarians and healthcare professionals that can be used for designing early detection and prevention strategies for managing Q fever. Ultimately, UHMthis study will aid in demonstrating that Q fever should not be neglected and should be included while controlling other zoonotic infections using the One Health approach.

1.4 Hypothesis

Coxiella burnetii infection is not highly prevalent among cattle in Narok County, Kenya

CHAPTER TWO: LITERATURE REVIEW

2.0 Aetiology of Q fever

Q fever is a zoonotic bacterial infection caused by *C. burnetii* a bacterial pathogen that has been found globally except in New Zealand (Tissot-Dupont and Raoult, 2008a). The disease was first reported in Queensland, Australia with concurrent detections in Montana, United States of America in the 1930's (Roest et al., 2013). *C. burnetii* is an obligate intracellular bacterial pathogen of the Coxiellaceae family that is distributed worldwide (Gürtler et al., 2014). The small gram negative bacteria is coccoid and pleomorphic existing in small, large and a spore-like state that persists in the environment (Angelakis and Raoult, 2010a; Gürtler et al., 2014). The spore like variant is the infectious state of *C. burnetii* and is very adaptive to various environmental conditions (Coleman et al., 2004; Gürtler et al., 2014).

Coxiella burnetii is exceedingly resistant to hostile physical environments and chemical agents; it can persist for months to years in the environment where it has been deposited (Angelakis and Raoult, 2010a; Njeru et al., 2016b). In human and animal infections, it preferentially targets tissue resident macrophages and the blood circulating monocytes (Porter et al., 2011a; Njeru et al., 2016a). Two distinct variants of *C.burnetii* have been demonstrated to exist; the phase I and II bacterial antigenic forms that are differentiated antigenically from each other by the surface lipopolysaccharide (Tissot-Dupont and Raoult, 2008b; Porter et al., 2011a; Njeru et al., 2016a). Phase I antigenic form is the extremely infectious variant that is commonly detected in natural infections while phase II form is less virulent and is mostly detected in laboratory culture systems after serial passages (Njeru et al., 2016b).

Over 30 genotypes of *C. burnetii* have been described to exist; some with a worldwide distribution with others restricted to a particular geographical locality (Gürtler et al., 2014). The genotypes differ on their virulence and pathogenic abilities (Russell-Lodrigue et al., 2009) and have been classified based on the nucleic acid sequence analysis of different gene

regions that are plasmid inclusive.

2.1 Transmission of Q fever

Ticks from the genus Dermacentor, Haemophysalis and Rhipicephalus (Ni et al., 2020) are currently considered as the main reservoir of the disease pathogens in the environment, however their role in the epidemiology and transmission of the diseases is not clearly understood (Psaroulaki et al., 2006). *C. burnetii* is often detected in vectors such as hard ticks. Under field conditions Q fever is more airborne than vector-borne. Tick feces are highly infectious with one gram of the feces containing up to around 10^9 *C. burnetii* organisms (Gürtler et al., 2014). Transcutaneous vector transmission of *C. burnetii* has been documented with such event considered rare in human counterparts (Delsing et al., 2010b; Gürtler et al., 2014).

Inhalation of contaminated aerosol is the main transmission pattern for humans (Angelakis and Raoult, 2010a; Gürtler et al., 2014). Domestic animals are highly implicated in human infections as infected animals discharge high amounts of *C. burnetii* organisms. The infective organisms can be discharged in fetal membranes, placenta and amniotic fluid during abortion storms; they can also be discharged in feces, urine and milk (Njeru et al., 2016a; Johnson et al., 2019b). After discharge, *C. burnetii* organism may persist in the environment from 150 (Welsh et al., 1959) to 365 days (Kersh et al., 2013). Oral transmission in human through consumption of milk had been implicated as a route of transmission (Bell et al., 1950) and this has been confirmed over the years with 0% to 95% unpasteurized milk having *C. burnetii* isolates (Pexara et al., 2018) . Human sexual transmission of the disease has also been documented (Milazzo et al., 2001).

2.2 Q fever disease dynamics

2.2.1 Q-fever in Kenya

In Kenya, the epidemiology of Q fever is not clearly understood due the obvious neglect of the

disease by both the animal and human health systems and possibly due to the other major diseases and limited availability of the technical and financial capacity to permit significant epidemiological studies (Njeru et al., 2016b). The first clinical case in humans in Kenya was reported in 1952 in Nairobi where a human patient developed a febrile disease that was presumed as a viral pneumonia. By 1956 seventeen cases had been reported in the country (Harris, 1952; Njeru et al., 2016b).

Since the first cases in the 1950's, two outbreaks have been described in Kenya, one involving safari travelers in the year 2000 and another outbreak in Baringo in the former Rift Valley Province in 2014 where 6 deaths were documented (Potasman et al., 2000b; ZDU, 2014b). In 2008, a seroprevalence of 18% in sheep, 32% in goats and 28% in cattle was detected in a study carried out in western Kenya with *C. burnetii* organism being detected through polymerase chain reaction in some of the small ruminants that had given birth during the study period (Knobel et al., 2013b). The bacterium was detected in several tick species including *Rhipicephalus sanguineus* and R. *appendiculatus*, *Amblyomma variegatum*, *Boophilus decoloratus* with more species of *Haemophysalis leachi* (the yellow dog tick) showing presence of the bacterium. The latter may indicate that the African domestic dog could be playing a role in the epidemiology of the disease in the tropics (Heisch et al., 1962; Buhariwalla et al., 1996; Knobel et al., 2013b). Archived human sera in the same study area showed a 30.9% seroprevalence, a possible indication of high human exposure rates in the study area (Knobel et al., 2013b).

In a study concluded in 1968, a 35.8% seroprevalence was documented from archived sera from five provinces in Kenya (former Rift Valley, Western, Coast, Eastern and Central Provinces) with each recruited hospital documenting at least one positive case (Vanek and Thimm, 1976; Njeru et al., 2016a). High human exposure rates of 26.8% have also been detected in Tana River County (Mwololo et al., 2014b).

Recent serological studies done in Kenya have suggested that herd management practices influence the transmission dynamics of Q fever and drought and famine episodes have led to the closer entanglement of wildlife, domestic ruminants and human beings in these ecosystems (Larson et al., 2019).

2.2.2 Q fever in other parts of the world

Q fever as a world zoonosis has been a public health concern for over 50 countries and due to the poor prevalence reports, it remains a misunderstood disease (Hadush et al., 2016). A range of mammalian hosts inclusive of cattle, sheep and goats have been implicated in the disease transmission with ticks completing the triad. Disease outbreaks have been seen to upscale during summer and spring in European countries and have been reported to be endemic in every other country except New Zealand. The WHO serosurvey that had 1400 samples test negative declared New Zealand free of the pathogen and the speculation is that the tick species present, *Haemophysalis*, is an inefficient vector and the cold temperatures make it almost impossible for the pathogen to multiply. A lot of Q fever serological studies have been done around the world but disease quantification in domestic ruminants has been hard to quantify (Pexara et al., 2018).

2.3 Q fever infection in animals

In ruminants, Q fever mainly present as abortions, stillbirths, early delivery, and birth of weak newborns (Angelakis and Raoult, 2010b; Agerholm, 2013). These clinical signs appear to be more common and likely in sheep, followed by goats and less likely in cattle (Porter et al., 2011b). Cattle are largely asymptomatic with the disease presenting as infertility, inflammation of the uterus and mastitis with varying degrees of placentitis (Tissot-Dupont and Raoult, 2008b; Angelakis and Raoult, 2010b; Porter et al., 2011a). Ruminants rarely show respiratory or cardiac signs both through natural and experimental infections unlike humans (Porter et al., 2011b). Shedding of *C. burnetii* organisms occur during birthing where high amounts are shed but infected animals shed organisms in milk feces and urine (Guatteo et al., 2006; Porter et al., 2011b). Research has demonstrated that cattle are more likely to be chronically infected than sheep and subsequently persistently shed the bacteria as a result (Porter et al., 2011b).

2.4 Q fever infection in Humans

Occupational exposure to *C. burnetii* is a common source of infection for humans and this occurs most often in farmers, veterinarians, and other people who work in the livestock sector (Porter et al., 2011a; Njeru et al., 2016a). Due to the clinical polymorphism nature of Q fever, it is mainly underreported and under diagnosed in human populations (Porter et al., 2011b; de Lange et al., 2015; Njeru et al., 2016a). With a wide incubation period of up to 3 weeks, Q fever may present in either an acute or a chronic form in humans depending on pathogen or host factors that influence disease progression (Gidding et al., 2009b; Porter et al., 2011b).

In the acute form, up to 60% of cases may be asymptomatic or may present as self-limiting illness characterized by an influenza-like syndrome demonstrated by fever, lethargy, headache, and muscle pain (Welsh et al., 1959; Watanabe and Takahashi, 2008a). Acute form

when clinically expressed, is often accompanied by an uncharacteristic pneumonia or hepatitis or a combination of both (Watanabe and Takahashi, 2008a; Porter et al., 2011b). Pneumonia which is usually mild is a main symptom of Q fever in human (Porter et al., 2011b). Cases of the pneumonia progressing to an acute distress syndrome have been reported (To et al., 1996b; Watanabe and Takahashi, 2008b). Endocarditis has been reported in cases of infection that resulted to maternal fetal death (Tissot-Dupont and Raoult, 2008b; Delsing et al., 2010a). Since the organisms can be discharged in milk, breast feeding is contraindicated (Carcopino et al., 2007). Mortality as a result of the acute form of the disease is estimated at 2%, myocarditis being the cause of death (Fournier et al., 2001).

Over 5% of the infected population develop the chronic form where the disease persist for over 6 months (Fournier et al., 2001; Porter et al., 2011b). Endocarditis is the main manifestation of the disease in about 70% of the infected population, nevertheless other symptoms such as chronic hepatitis, chronic fatigue syndrome, septic arthritis, osteomyelitis and interstitial lung disease have been reported to occur (Fenollar et al., 2004; Hickie et al., 2006). Q fever associated endocarditis accounts to about 3% of the total human endocarditis cases (Fenollar et al., 2004; Parker et al., 2006; Porter et al., 2011b). Neurological signs have also been reported in the chronic form of the diseases with subsequent spontaneous abortions being reported in pregnant women (Bernit et al., 2002b; Porter et al., 2011b). Treatment with antibiotic is rarely successful with disease mortalities reaching up to 50% (Watanabe and Takahashi, 2008b).

2.5 Risk factors associated with transmission of Q fever

In animals, Q fever seroprevalence differs broadly by animal species and their topographical location with the risk factors that result in this variability in seropositivity being poorly understood (Vanderburg et al., 2014b). Nevertheless, Intrinsic management and production

systems may potentiate infections in herds (Georgiev et al., 2013). A link between the number of positive animals in a farm and poor management systems such as lack of quarantine and health checks in animals introduced in the herd has been shown (Georgiev et al., 2013).

Cattle and sheep that have had previous abortions before have had higher seroprevalence as compared to those that have never experienced abortions (Esmaeili et al., 2019). Dairy cows, ewes and does are more likely to have *C. burnetii* isolated from then than their male counterparts in the same farm and this is thought to be due to the milking as a shedding route (Nokhodian et al., 2016)

Livestock raised in wildlife corridors are more times likely to be *C. burnetii* positive than intensively raised livestock in agro-farmer zones (Nguro, 2017)

Q fever is largely an occupational disease with people who are in close contact with animals (animal owners, workers and veterinarians) more likely to contract the disease as opposed to the general population (de Lange et al., 2015; Njeru et al., 2016b). Individuals who are in contact with small ruminants are more likely to be seropositive for *C. burnetii*. This has been elaborated in various studies where farmers owning sheep and or goats have been found more likely to contract the disease as opposed to cattle ownership (Rooij et al., 2012; Schimmer et al., 2012; Boden et al., 2014; de Lange et al., 2015). Since inhalation of infective dust particles is largely involved in the transmission of the disease, individuals and households proximal to an infected farm also stand a high risk of being infected (Georgiev et al., 2013); nevertheless geographical landscape and environmental/climatic conditions such as sandstorms and dry weather play a role in proximity to farm as a risk factor of transmission. In some outbreaks, involvement of other animals other than ruminants increases the risk of seropositivity in humans. Mixed farms with cats, deer, dogs and pigeons predispose human to higher contact rates to infected fecal animal material from these companion animals

(Schimmer et al., 2012; Georgiev et al., 2013). It has been demonstrated that of wearing gloves and respiratory masks during and around lambing/kidding protects animal handlers against *C. burnetii* infections (Schimmer et al., 2012).

2.6 Diagnosis of Q fever

Diagnosis of the Q fever can be achieved through direct methods aimed at identifying the presence of the bacterium or some of its components or through indirect methods. One of the methods of direct detection is through staining and visualization of smears. This method is not however reliable as there is possible confusion with other organisms such as *Chlamydia* and *Brucella* species (Guatteo et al., 2006). *Coxiella burnetii* can also be cultured like other bacteria and bacterial culture is the gold standard for detection of bacteria colonies (Omsland et al., 2009; Porter et al., 2011b). The organisms can be cultured competently in the yolk sac of chicken embryos among other diverse cellular tissues (Omsland et al., 2009). This method is however not highly used as there is always a need of a biosecurity level 3 facility for this process in addition to the method having low sensitivity (Porter et al., 2011b; Njeru et al., 2016b). Immunohistochemistry and Polymerase chain reaction can also be used on (Fournier and Raoult, 2003; Porter et al., 2011b).

Indirect diagnostic techniques identify specific humoral or cellular immunity responses to *C. burnetii* infections (Porter et al., 2011b). Histopathology can also be used in diagnosis of the diseases but lesions seen in cases of Q fever are not pathognomonic (Bildfell et al., 2000; Porter et al., 2011b).

2.6.1 Serological techniques for Q fever diagnosis

There are 3 main serological techniques employed in the diagnosis of Q fever, these being complement fixation, indirect immunofluorescence and enzyme linked immunosorbent assay (ELISA).

Other methods include micro agglutination, dot immunoblotting, western immunoblotting, indirect hemolysis test and radioimmunoassay. These techniques are not commercially available for Q fever and therefore have been limited in their use.

Complement fixation test (CFT) was the reference serological diagnostic technique in veterinary medicine (Porter et al., 2011b). The method has become less popular than indirect immunofluorescence assay and ELISA which are more sensitive, specific and less laborious (Péter et al., 1985; Rousset et al., 2007). CFT has a higher specificity than sensitivity and is used to detect both phase one and two *C. burnetii* antibodies (Fournier et al., 1998).

Indirect immunofluorescence assay (IFA) is the simplest, highly specific method used to detect antibodies. However, the process is time consuming since it requires the production of highly sensitive *C. burnetii* phase 1 antigen that are harvested from mice spleens after inoculation with Phase 2 antigens.

ELISA was fronted as good seroepidemiological tool by Field et al., (2000) after he compared results from ELISA and IFAT and presented the former to have 99% sensitivity and 88% specificity without rheumatoid factor cross-reactivity. ELISA has been shown to be in agreement with the reference test IFAT over the years during Q fever testing. The test employs the principle of measuring the degree of color development and correlating it to the amount of antibodies present in the test serum. It detects the presence phase II IgG antibodies.

These indirect methods have a disadvantage as they over rely on specific antibodies which are absent up to three weeks from infection, making early detection of disease a challenge (Kuroiwa et al., 2007; Porter et al., 2011b).

2.6.2 Other diagnostic methods

Polymerase Chain Reaction (PCR), culture from affected tissue such as aborted fetus, placenta and immunohistochemistry have been shown to confirm tentative *C. burnetii* infections ("Diagnosis and Management of Q Fever — United States, 2013," n.d.). PCR has been recommended by the OIE as the new era method to confirm acute infections and many countries are currently developing in-country protocols and kits (Marushchak et al., 2019)

2.7 Control and Treatment of Q fever

2.7.1 Q fever control in animals and humans

Control strategies aimed at the disease in animals are key in significantly decreasing the incidence of Q fever in humans (Njeru et al., 2016b). Unfortunately, there are no licensed vaccines for use in the control of the disease in animals but a partially licensed inactivated phase I vaccine is currently used to control the disease in Europe (Porter et al., 2011b). Human vaccines developed against the disease have equally not been successfully used in controlling the disease, subunit vaccine or live attenuated strain vaccines that have are used have serious adverse reactions in disease exposed individuals (O'Neill et al., 2014). An Australian licensed formalin killed whole-cell vaccine is effectively used in high risk groups in both animals and humans in Australia however, both of these animal and human vaccines are not available in Kenya (Njeru et al., 2016b).

2.8 Q fever chemotherapy in animals and humans

In animals appropriate antibiotic therapy (doxycycline) has been found to significantly aid in acute infections whilst the chronic state has been shown to require continuous serological surveillance and monitoring on top of the prolonged antibiotic use (Woldehiwet, 2004).

If doxycycline is contraindicated due to allergies second and third generation antibiotics may be considered trimethoprim/sulfamethoxazole, moxifloxacin and clarithromycin (Jones et al., 2006).

Measures for the control of animal Q fever include prophylactic antibiotic administration during dry cow periods (Astobiza et al., 2013), isolated kidding area, proper management of fecal and wool shearing waste, practice of good vaccination protocols, biosecurity limitations, and vector control (Porter et al., 2011b) According to the (OIE, 2010) gravid livestock should be culled and issuing of bull breeding bans in farms with outbreaks whilst controlling animal movements are recommended.

Treatment of Q fever is mainly done in humans by use of antibiotics through prolonged dosages of any of these antibiotics; Rifampin, doxycycline, erythromycin, cotrimoxazole clarithromycin, and or roxithromycin (Porter et al., 2011b). This may be combined with other supportive treatment and is custom made to each patient physiological and disease progression (Porter et al., 2011b).

2.9 Drug resistance Q fever

Coxiella burnetii is most susceptible to tetracyclines in animals although other drug have been used such the fluroquinolones and with some strains of the bacteria have been rendered resistant (Vranakis et al., 2010). Low levels of resistance have been associated with Fluoroquinolone therapy, the hypothesis being an in body resistance or gyrase A mutations.

CHAPTER THREE: MATERIALS AND METHODS

3.0 Study area

The study samples were obtained from Maasai Mara ecosystem in Narok County, Kenya (Figure1). Maasai Mara is catalogued in the country's arid and semiarid lands. Farm animal production and wildlife tourism are the main livelihood supporting domains covering 25,000km^2 from northern Kenya extending to the neighboring country Tanzania. The main inhabitants of this ecosystem include are the Maasai pastoralists who still raise and manage their cattle through extensive grazing systems. Based on land utilization patterns; open range grazing and plant crop production are the main enterprises which occur in closeness to the wildlife corridor reserve. The ecosystem was divided into 3 zones for purposes of this project. Zone one was next to the Masai Mara National Reserve and this was anticipated to have high wildlife-cattle interactions, zone two was a mixed area mostly within or next to community conservancies with moderate wildlife-cattle interactions and zone three on the outer ring mainly characterized with very little wildlife-cattle interactions. These three zones were used during the analysis of risk factors associated with *C. burnetti* seroprevalence as they had different levels of wildlife–livestock interactions.

Selection of villages

Five villages were purposely selected for the study after participatory epidemiology exercises with the local stakeholders to categorize villages into the three zones.

3.1 Study design

This was a cross-sectional study with multistage sampling that was conducted between January 2020 and March 2020. All the sera samples collected were stored at the International Livestock Research Institute at -310 degrees Fahrenheit in a cryogenic bank. The total number of animals sampled per zone was estimated

using the formula: n = (1.96)2p (1 -p)/d2, with a margin error (d) of 0.05 (Dohoo et al., 2003).

Prevalence of *C. burnetii* infections estimated in pastoral systems was 26% (Oboge *et al.*, 2016)

Based on formula by Naing et al, 2006; Dohoo et al, 2003)

$$n = Z2 \frac{(P)(1-P)}{d2}$$

Where: n = Sample size Z = Z value (Confidence level, e.g. 95%)

P = estimate of the proportion or anticipated prevalence d = confidence interval or the required precision

n= 296

To adjust for intra-herd correlation, an intra-cluster correlation of 0.5 using the formula

Design effect=1+ICC (K-1)

K= average number of animals per herd= 3; ICC = 0.5 gives a design effect of 2 296x2=592

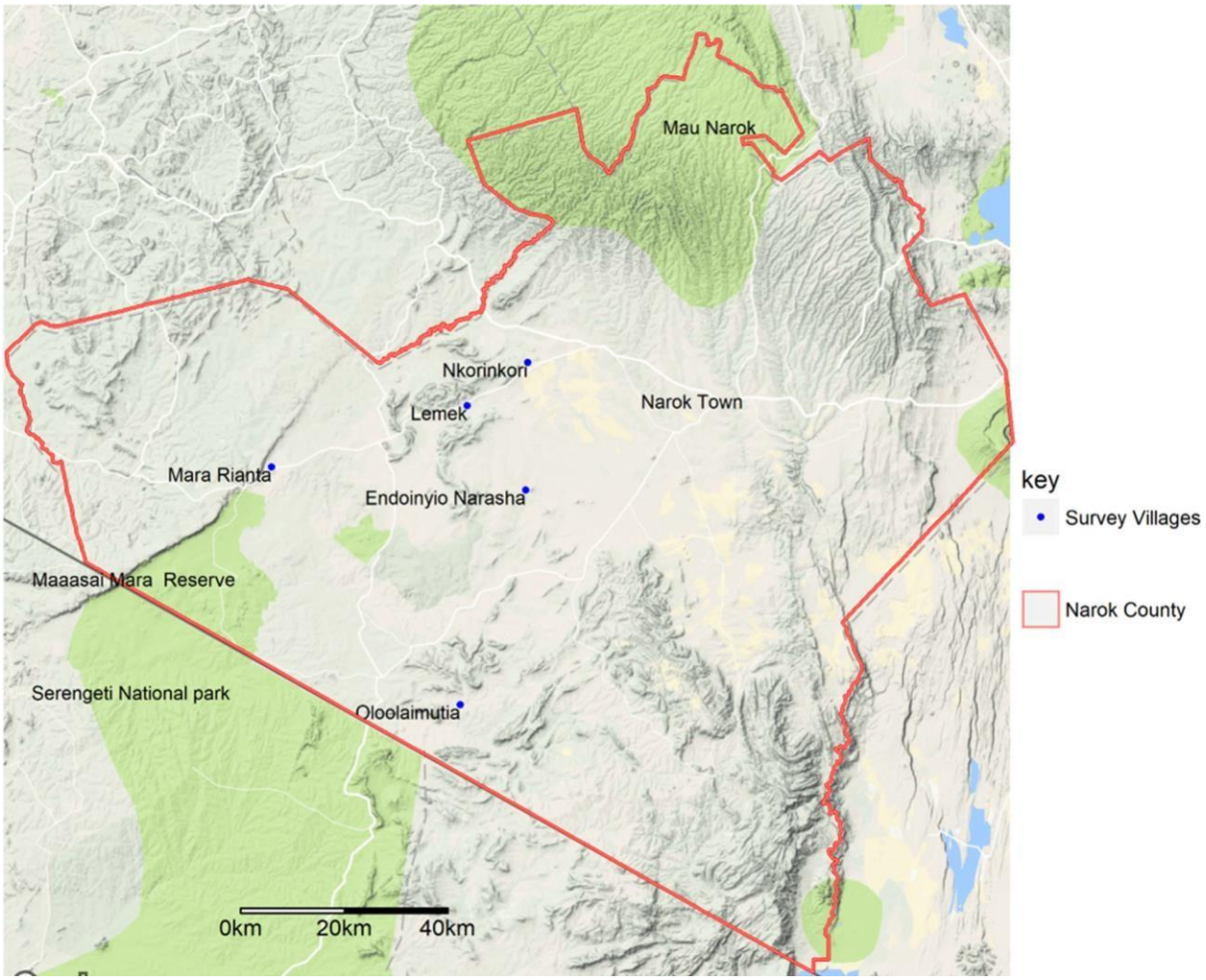

Figure 3.1: Sampling area within the Mara ecosystem in Narok County

3.2 Determination of sero-prevalence

This study used stored samples from ILRI'S Repository laboratory described above. These serum samples were from a previous study (Zoonotic Pathogen Seroprevalence in Cattle in a Wildlife-Livestock Interface, Kenya) done in the Mara region by Nthiwa et al., (2019). A commercially-available enzyme-linked Immunosorbent assay (ELISA) kit (IDEXX) was used for the detection of phase II IgG antibodies against *C. burnetii* according to the manufacturers' instructions. The assays were performed at the ILRI laboratory in Nairobi. The test kit employed the principle of measuring the degree of color development and correlating it to the amount of antibodies present in the test serum.

Serum samples randomization and identification

The batch samples that had been collected by Daniel Nthiwa et al., 2019, were 1100 in number and based on sample calculation, this prevalence study needed 592 samples. The serum samples had been collected from three zones characterized by the wildlife interaction intensity where zone 1 (Mara Rianta and Oloolaimutuia) was high interface area with maximum cattle wildlife interaction, zone 2 (Lemek and Endoinyio Narasha) was medium interface region and zone 3 (Nkorinkori) was low interaction of cattle with the wildlife.

Each zone had then subjected into herding ratios where zone 1 had 155 herds, zone 2 had 155 herds and lastly zone 3 had 80 herds. Maintaining this ratio with a finite sample population of 592; zone 1 and 2 were allocated 236 samples each while zone 3 was allocated 128 samples. Each zone serum list was subjected to excel randomization and the first 236 sample ids were picked for both zones 1 and 2 and the first 128 sample Ids for the zone 3.

Reagent and test serum preparation

Using the appropriate laboratory practices, the wash concentrate was diluted 1:10 using distilled water. The serum samples needed to be diluted to 1:400 using the wash. The 589 serum samples were thawed at -80°C then at -20°C then at room temperature to allow well-paced complete thawing. These serum samples were then subjected to vortexing to allow a homogenous output. In order to obtain working dilutions of the test sera, neat serum samples were diluted serially with a diluent as described below. At first, 6µl serum was diluted with 114 µl of the diluent to give a final dilution of 1:20 ratio. Following a thorough mixing, another 6µl of the diluted serum was dispensed into another well and then further diluted with 114µl of diluent to get a final dilution ratio of 1:400. This process of the serial dilution was also done for both the negative and positive controls sera.

The ELISA (IDEXX) test was performed according to the manufacturer's instruction. It is a commercial kit that may be used to test a large amount of sera and usually the serological test of choice better suited was Indirect Fluorescent antibody test (IFAT). The IDEXX kit came with 8 packed reagents. These included 2 pre-coated antigen plates, conjugate, tetramethylbenzidine substrate (TMB), stop solution, wash concentrate, positive and negative controls.

Analysis of sera for *Coxiella burnetii* antibodies by ELISA

One hundred microlitres of test sera dilutions were then pipetted into the microtiter plate wells coated with *C. burnetii* antigens in duplicates where a complex was expected to form after gentle tapping and one hour incubation at 37^0C. The antibodies present (if any) were expected to bind to the phase I or phase II antigen to form an antigen-antibody complex (just like what forms in-vivo during an active infection).

The next step involved washing with 300µl of wash solution three times to remove any unbound material and the plate was then dried on absorbent paper. One hundred microliters of peroxidase labelled anti-ruminant IgG was added to be bound to the already formed antigen-antibody complexes and incubated again for 60 minutes at the same temperature as the initial incubation and soon after a three time repeat wash using 300µl of wash solution was done. Washing was done three times using a wash buffer in a multi-channel washer to make sure that any protein or antibody that was not bound to its target was removed. Thereafter, a 100µl of TMB substrate was dispensed in to the microliter-wells followed by incubation at room temperature in a dark area for 15 minutes. The final step involved the addition of 100µl of stop solution into these wells to stop the reaction.

All these steps were timed and the right incubation temperature adhered to whilst observing strict measuring and pipetting to maintain precision and accuracy. The results were then obtained by comparing the optical density (OD) value of the sample wells with OD value of

the positive control well. The plate was taken to a plate reader where the machine was set to read both at 450nm and 650nm and the output was deduced by the reader software.

Sera with OD value ≥40% were defined as positive and values < 30% were considered as negative.

Where this calculation OD = 100 × (OD value of test sample – OD value of negative control)

Calculations

NCX-{NC1A (450) +NC2A (450)} 2; this was the negative control average

PCX-{NC1A (450) +NC2A (450)} 2; this was the positive control average

Validity of criteria was guided by NCX ≤ 0.5; PCX ≤ 2.5 and PCX-NCX ≥ 0.3

For a sample to be considered positive or negative : the negative control average was subtracted from sample OD at 450nm then divided by the difference in negative control average from positive control average then multiplied by 100 to give a percentage. If the sample percentage was < 30% then it was determined negative, if ≥ 40% it was determined positive and in between it was considered suspect where a second repeat of the test was done. The ODs were not converted to percentage proportions.

The formula is S/P%= sample A (450)-NCX A (450)/ (PCX-NCX)

IDEXX Kit-Q fever Validation

Two plates were run to see how the optical densities compared with the negative and positive control optical densities and the output was valid. This then gave lee-way for the testing of the 589 serum samples using the above described dilution and test protocols.

Data on risk factors associated with Q fever seroprevalence

A questionnaire was administered to the animal owners during the collection of samples. The

data collected was believed to influence the transmission and prevalence of *C. burnetii*. The data used were the sex of the animals, herd size, herd management practice, i.e. (use of bull or artificial insemination), utilization of communal grazing areas, grazing practices around the wildlife reserves, mixture of cattle herds during grazing and during watering, introduction of newly bought cattle and the clinical signs observed both in animals and the people handling the livestock.

3.4 Data management and statistical analyses

Data from the household questionnaire survey and serological tests were entered and saved in Microsoft Office Excel 2013 Windows 10. The data was then screened for any entry errors and imported into R studio. Descriptive statistics were performed, where proportions, means and medians were calculated; the variables involved were zones, villages, animal herd characteristics and management. Association between and among levels of these categories were analyzed using multilevel modelling adjusting for either animal or herd level factors in each model.

The outcome variable was the exposure status to *C .burnetii* antibodies and was defined as categorical dichotomous (two-level with a yes/no option). The sample data was collected randomly hence the test for normality was not done. Univariable and multivariable logistic analysis using R 3.84 software was further carried out at animal and herd level to determine odd's ratio (OR) at 95% confidence intervals for the following dependent variables: zoning structure, sex, cattle herd size, herd management practice, communal grazing, grazing in the wildlife reserve, shared watering points, contact with cattle from different villages, new livestock bought the previous year, ownership of bulls and abortions in the herds.

Univariable logistic regression analysis was done using the GLMR function in the LME4 model package (R.3.84 software). After fitting factors that had a P-value of < 0.2 they were then selected for multivariable analysis as recommended in Veterinary Epidemiologic research

3rd edition (Dohoo et al., 2009). These factors were considered significant.

Multivariable logistic regression was done after the maximal model fitting, where all variables associated with exposure to *C. burnetii* antibodies with a P-value of <0.05 were considered significant covariates. In order to assess the fit the plot of the model was inspected. This selection followed the forward and backward procedure. The intra-cluster correlation was extracted using the variant components from the multivariable logistic regression analysis.

CHAPTER FOUR: RESULTS

4.0 Zone, village and animal demographics

Sample distribution by zones and villages

Out the tested samples, 77.9% (452/589) were from cows and 22.1% (130/589) from bulls (Figure 4.1). The herd size ranged from 1-390 with a mean herd size of 197, median herd size of 200 and a mode of 377. The proportion of sample tested among the 5 villages were as follows: Mara Rianta 18.7% (110/589), Oloolaimutia 20.2% (119/589), Endoinyio Narasha 19.5% (115/589), Lemek 21.1% (124/589) and Nkonrinkori 20.5% (121/589) (Figure 4.2). These villages were zoned into 3 and the proportion of samples tested for zone 1-High wildlife-farm animal interface area (Mara Rianta and Oloolaimutia) was 38.9% (229/589), zone 2 medium wildlife-farm animal interface area (Endoinyio Narasha and Lemek) was 40.6% (239/589) and zone 3 low wildlife-farm animal interface area (Nkonrinkori) was at 20.5% (121/589) (Figure 4.3).

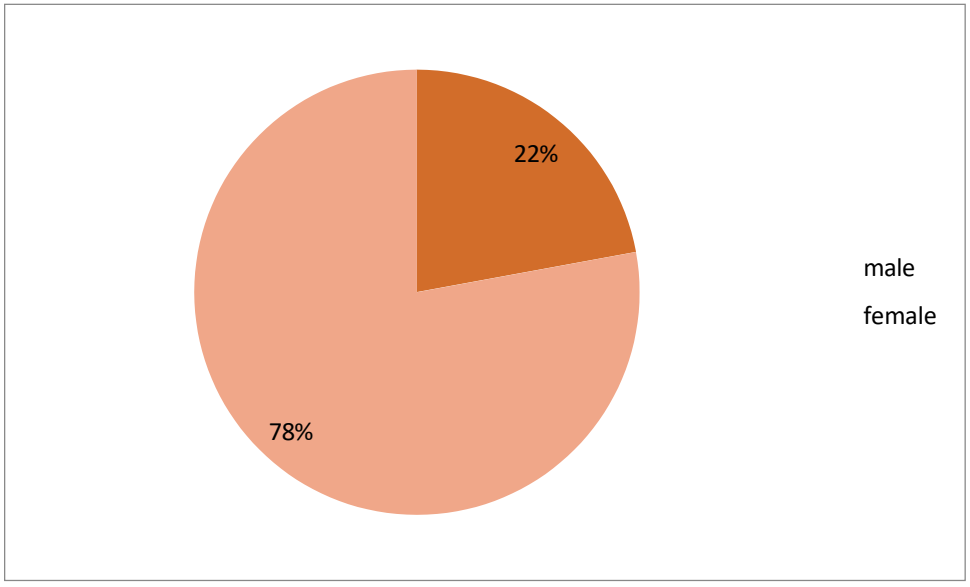

Figure 4. 1: Proportions by the sex of the animals for the selected samples

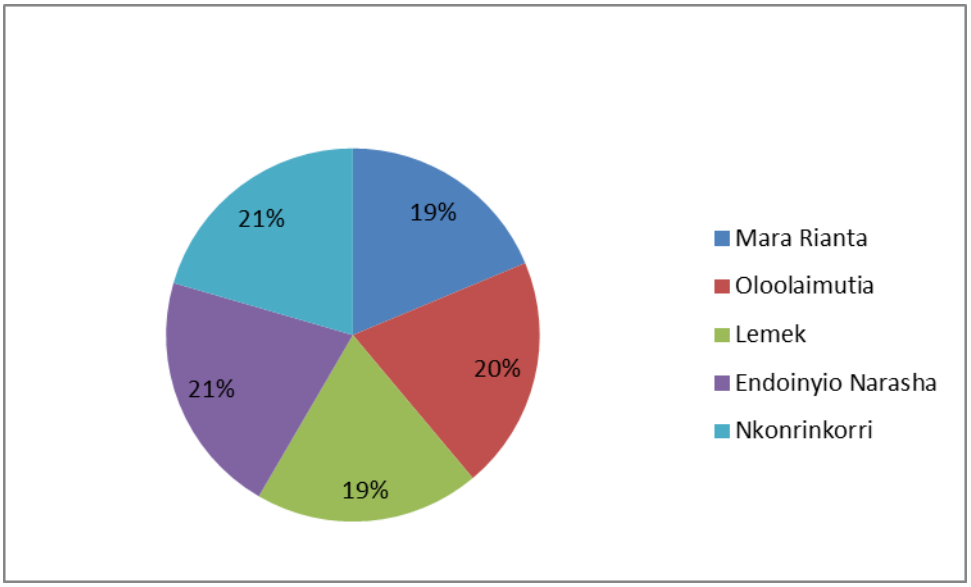

Figure 4. 2: The proportion of animal samples used from each village

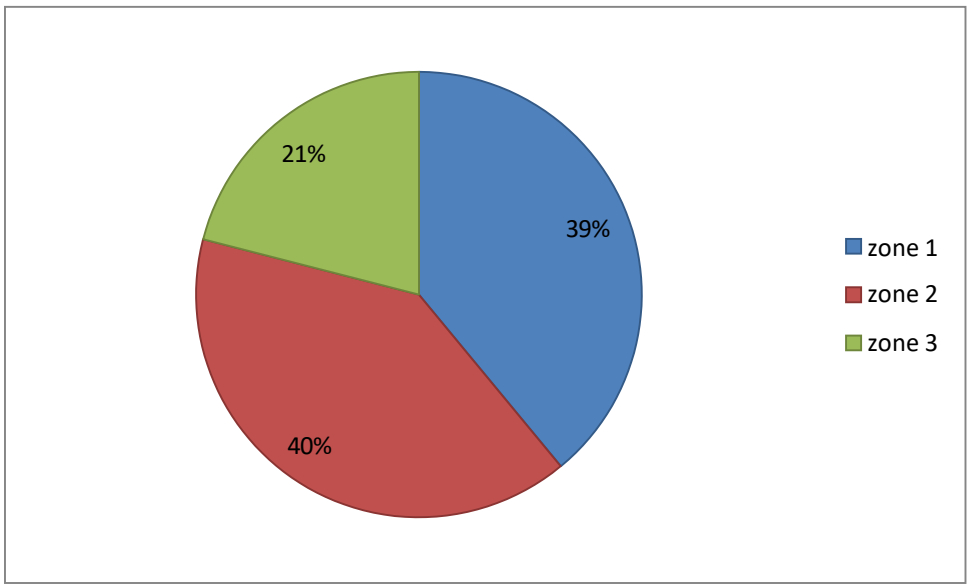

Figure 4. 3: The proportion of the samples tested by zonal distribution

4.2 Animal characteristics and management

The risk factor percentage proportions after descriptive analysis were shown as table 4.2 below, with each variable category percentage being calculated over the total n (589). The female animals sampled were higher in number than the male with a percentage of 77.9% (459/589). The moderate interface zone (2) had a higher percentage sampled with 40.6 % (239/589) as zone (1) had 38.9% (229/589) and zone (3) had 20.5% (121/589). Cattle with herds over 50

had higher sample proportions than those with less than 49 animals per herd at 58.1% (342/589). Those animals that grazed near wildlife reserves had higher samples number than those further away at 56.7%. The animals that were in mixed during herding had higher sample proportions at 84.7% (499/598), those that shared water points between villages at 57% (336/589) and shared water points within villages at 95.4% (562/589). Herds with previously bought livestock had higher sample numbers than those without at 73.2% (431/589) and those herds that had experienced abortion at 50.6% (298/589)

Table 4. 1: Distribution of animal characteristics and management in Maasai Mara

Variable	Category	Number of animals tested	Percentage Proportions
Animal sex	Male	130	22.1%
	Female	459	77.9%
Zones	Zone 3 (low interface area)	121	20.5%
	Zone 2 (moderate interface area)	239	40.6%
	Zone 1 (high interface area)	229	38.9%
Cattle herd size	≤ 49 cattle	247	41.9%
	>50 cattle	342	58.1%
Herd management practice	Sedentary	334	56.7%
	Pastoral	255	43.3%
Communal grazing	No	436	74.0%
	Yes	153	25.9%
Village graze - sharing grazing areas within the village	No	72	12.2%
	Yes	517	87.8%
# other Village graze - sharing grazing areas between villages	No	440	74.7%
	Yes	149	25.3%
Cattle grazing in wildlife	No	334	56.7%

reserves	Yes	255	43.3%
Inter-herd mixing when grazing	No	90	15.3%
	Yes	499	84.7%
Shared water points between villages	No	253	42.9%
	Yes	336	57.0%
Shared water sources within villages	No	27	4.6%
	Yes	562	95.4%
Bought livestock in previous year	No	158	26.8%
	Yes	431	73.2%
Bull ownership	No	90	15.3%
	Yes	499	84.7%
Abortions in herd previously	No	291	49.4%
	Yes	298	50.6%

4.3 Seroprevalence of *Coxiella burnetti* among cattle

Out of the 589 serum samples tested using IDEXX Elisa Q fever kit only 11 samples tested positive for *C. burnetii* giving an apparent prevalence of 1.87% (Table 4.2). Male animals' seropositivity was estimated at 3.08% (95% CI 0.85 ± 7.69) while female animals' seropositivity was at 1.53% (95% CI 0.62 ±3.11) and this was not significantly different (Table 4.2). Zonal sero-positivity grouped according to wildlife interaction intensity had low interface area (zone 3) at 2.48% (95% CI 0.51 ± 7.07), moderate interface area (zone 2) at 1.67% (95% CI 0.46 ± 4.23) and high interface area (zone 1) at 1.75% (95% CI 0.48 ± 4.42). There was no significant difference between the sero-positivity across the zones based on the CIs. Based on

cattle herd size, the herds with ≤ 49 cattle had a sero-positivity estimated at 1.69% (95% CI 0.44 ± 4.09) while those with >50 cattle had a sero-positivity of 2.05% (95% CI 0.83 ± 4.17) and these were not significantly different. These villages practiced either a sedentary or pastoral herd management where the former had a sero-positivity of 1.80% (95% CI 0.66 ± 3.870) and the latter had 1.96% (95%CI 0.63 ± 4.51) and these were not significantly different. Those that practiced communal grazing showed a C. *burnetii* positivity of 2.61% (95% CI 0.72 ± 6.56) in their animals as opposed to those who did not at 1.6% (95%CI 0.65 ± 3.28). Cattle that was grazed within one village had sero-positivity of 1.93% (95% CI 0.93 ± 3.52) while those that shared grazing areas between villages had 2.68% (95% CI 0.73 ± 6.73).Cattle grazed in wildlife reserves had a seropositivity of 1.57% (95% CI 0.43 ± 3.97) as opposed to the animals that were not grazed there at 2.09% (95% CI 0.85 ± 4.27). Animals from different herds that mixed had a percentage positivity of 1.60% (95% CI 0.69 ± 3.13) as opposed to the herds that never mixed at 3.3% (95% CI 0.69± 9.43).The next variable category are those animals that shared water points between the 5 different villages had the percentage at 2.08% (95%CI 0.84 ± 4.25), those that shared water sources within individual villages at 1.95% (95% CI 0.98 ± 3.48). Those herds that newly introduced livestock to their herds in the previous year showed a significant result of 2.32% (95% CI 1.12 ± 4.22) as opposed to those herds that did not have any new animals at 0.63% ((95% CI 0.16 ± 3.48). Another significant result was the herds serviced with an in-house bull showing a lower sero-positivity at 1.20% (95% CI 0.44 ± 2.59) compared to those herds that used borrowed bulls from elsewhere at 5.55% (95% CI 1.83 ± 12.49).The herds that had experienced abortions before showed a seropositivity of 1.00% (95% CI 0.21± 2.91) and those that did not at 2.75% (95% CI 1.19 ± 5.34)

Table 4. 2 Zonal, Management and animal factors associated with seropositivity to *Coxiella burnetii* in Maasai Mara

Variable	Category	Number of animals tested	Seropositivity (%) (95% CI)
Sex	Male	130	3.08 (0.85 – 7.69)
	Female	459	1.53 (0.62-3.11)
Zones	Zone 3	121	2.48 (0.51-7.07)
	Zone 2	239	1.67 (0.46-4.23)
	Zone 1	229	1.75 (0.48-4.42)
Cattle herd size	≤ 49 cattle	247	1.69 (0.44-4.09)
	>50 cattle	342	2.05 (0.83-4.17)
Herd management practice	Sedentary	334	1.80 (0.66-3.870)
	Pastoral	255	1.96 (0.63-4.51)
Shared a communal grazing area	No	436	1.61(0.65-3.28)
	Yes	153	2.61 (0.72-6.56)
Grazing areas shared within the village	No	72	1.39 (0.04-7.5)
	Yes	517	1.93 (0.93-3.52)
Grazing areas shared between villages	No	440	1.59 (0.64-3.25)
	Yes	149	2.68 (0.73-6.73
Graze cattle in wildlife reserves	No	334	2.09 (0.85-4.27)
	Yes	255	1.57 (0.43-3.97)
Mix cattle with	No	90	3.33 (0.69-9.43)

others from different herds during grazing	Yes	499	1.60 (0.69-3.13)
Shared watering points between villages	No	253	1.58 (0.43-3.99)
	Yes	336	2.08 (0.84-4.25)
Shared water sources within villages	No	27	0 (0-1.28)
	Yes	562	1.95 (0.98-3.48)
Bought livestock in the previous year	No	158	0.63 (0.16-3.48)
	Yes	431	2.32 (1.12-4.22)
Bull ownership	Own bull	90	5.55 (1.83-12.49
	Bull from another farm	499	1.20 (0.44-2.59)
History of abortions in the sampled herds	No	291	2.75 (1.19-5.34)
	Yes	298	1.00 (0.21-2.91)

Factors associated with seropositivity in univariable and multivariable logistic regression analysis. The factor associated with a significant outcome variable (positive or negative to *C. burnetii* antibodies) at the animal level was bull ownership. The herds that were serviced by their won bull had a lower *C. burnetii* sero-positivity level compared to those herds that used a borrowed bull for breeding.Those associated with significant outcome variables at herd level were communal grazing, animals sharing grazing areas between villages, inter-herd mixing when grazing, bull ownership, herds with previously purchased livestock and abortions in the herd.

The intra-cluster correlation (ICC) at the herd level was 0.00.

– Animal and herd level variables investigated for association with *C. burnetii* seroprevalence using univariable logistic regression.

Animal-level factors

At the animal level, bull ownership factor was associated ($P<0.05$) with *C. burnetii* seropositivity based on univariable analysis (Table 4.3). Herds with borrowed bulls from other farms were more likely to be seropositive compared to those herds that used their own farm bull (95% CI 0.4 ± 16.2); P= 0.016).

Table 4. 3: Variables investigated for their association with animal-level seropositivity of *Coxiella burnetii*

Variable	Category	No. of animals tested	% seropositive (95% CI)	Odds Ratio (95% CI)	P value
Sex	Male	130	3.08 (0.85 – 7.69)	1 (Ref.)	
	Female	459	1.53 (0.62-3.11)	0.49 (0.14-1.69)	0.26
Zones	Zone 3	121	2.48 (0.51-7.07)	1 (Ref.)	
	Zone 2	239	1.67 (0.46-4.23)	0.7 (0.1-3.0)	0.60
	Zone 1	229	1.75 (0.48-4.42)	0.7 (0.2-3.2)	0.64
Cattle herd size	≤ 49 cattle	247	1.69 (0.44-4.09)	1 (Ref.)	
	>50 cattle	342	2.05 (0.83-4.17)	1.3 (0.4-4.4)	0.706
Herd management practice	Sedentary	334	1.80 (0.66-3.870)	1 (Ref.)	
	Pastoral	255	1.96 (0.63-4.51)	1.1 (0.3-3.6)	0.884
Shared a communal grazing area	No	436	1.61(0.65-3.28)	1 (Ref.)	
	Yes	153	2.61 (0.72-6.56)	1.6 (0.5-5.7)	0.432
Grazing areas shared within the village	No	72	1.39 (0.04-7.5)	1 (Ref.)	
	Yes	517	1.93 (0.93-3.52)	1.4(0.2-11.1)	0.75
Grazing areas shared between villages	No	440	1.59 (0.64-3.25)	1 (Ref.)	
	Yes	149	2.68 (0.73-6.73	1.7 (0.5-5.9)	0.399
Graze cattle in wildlife reserves	No	334	2.09 (0.85-4.27)	1(Ref)	
	Yes	255	1.57 (0.43-3.97)	0.7(0.2-2.6)	0.641

Mix cattle with others from different herds during grazing	No	90	3.33 (0.69-9.43)	1 (Ref.)	
	Yes	499	1.60 (0.69-3.13)	0.5 (0.1-1.8)	0.275
Shared watering points between villages	No	253	1.58 (0.43-3.99)	1 (Ref.)	
	Yes	336	2.08 (0.84-4.25)	1.3 (0.4-4.6)	0.657
Shared water sources within villages	No	27	0 (0-1.28)	1 (Ref.)	
	Yes	562	1.95 (0.98-3.48)	Infinite	0.890
Bought livestock in the previous year	No	158	0.63 (0.16-3.48)	1 (Ref.)	
	Yes	431	2.32 (1.12-4.22)	3.7 (0.5-29.4)	0.211
Bull ownership	Bull from another Farm	90	5.55 (1.83-12.49	4.8 (0.4-16.2)	0.016
	Own bull	499	1.20 (0.44-2.59)	1 (Ref)	
History of abortions in the sampled herds	No	291	2.75 (1.19-5.34)	1 (Ref.)	
	Yes	298	1.00 (0.21-2.91)	0.4 (0.1-18.4)	0.134

Herd level factors

At the herd level univariable analysis; 6 factors were associated with the outcome variable (Table 4.4). Cattle that had common grazing areas were more likely to be *C. burnetii* seropositive with an odds ratio (OD) 2.2 (95% CI 1.0 ± 4.8); P value 0.05. Cattle that shared grazing areas between villages were more likely to be seropositive OD 2.3 (95% CI 1.0 ± 5.0); P value 0.04. Animals in different herds that mixed during grazing were more likely to be positively associated with C. *burnetii* OD 0.4 (95% CI 0.2 ± 0.9) P value 0.029. Herds with newly purchased livestock from the previous year were more likely to be positively associated with the outcome variable OD 10.1 (95% CI 1.3 ± 75.8); P value 0.025. Farms that were serviced with bulls from different farms were more likely to be associated with the outcome variable OD 4.5 (95% CI 2.0 ± 10.3); P value 0.0003. Herds that had already experienced abortions were more likely to be associated with the outcome variable OD 0.3 (95% CI 0.1 ± 10.9); P value 0.018 (Table 4.4).

Table 4. 4: Variables investigated for their association with herd-level seropositivity of *Coxiella burnetii.*

Variable	Category	No. of animals tested	% seropositive (95% CI)	Odds Ratio (95% CI)	P value
Sex	Male	130	3.84 (1.26 – 8.74)	1(Ref.)	
	Female	459	4.57 (2.85- 6.90)	1.2(0.4-3.2)	0.721
Zones	Zone 3	121	4.96(1.84-10.48)		
	Zone 2	239	4.18(2.02-7.56)	0.8(0.3-2.4)	0.737
	Zone 1	229	4.37 (2.11-7.88)	0.9 (0.3-2.5)	0.801
Cattle herd size	≤ 49 cattle	247	3.24(1.41-6.28)	1(Ref)	
	>50 cattle	342	5.26(3.15-8.19)	1.7(0.7-3.9)	0.242
Herd management practice	Sedentary	334	3.89(2.08-6.56)	1(Ref)	
	Pastoral	255	5.10(2.74-8.56)	1.3(0.6-2.9)	0.482
Shared a communal grazing area	No	436	3.44(1.94-5.61)	1(Ref)	
	Yes	153	7.19(3.64-12.50)	2.2(1.0-4.8)	0.057
Grazing areas shared within the village	No	72	2.78(0.34-9.67)	1(Ref)	
	Yes	517	4.64(2.99-6.82)	1.7(0.4-7.4)	0.476
Grazing areas shared	No	440	3.41(1.92-5.56)	1(Ref)	

between villages	Yes	149	7.38(3.74-12.83)	2.3(1.0-5.0)	0.0463
Graze cattle inwildlife reserves	No	334	4.79(2.76-7.66)	1(Ref)	
	Yes	255	3.92(1.90-7.09)	0.8(0.4-1.8)	0.612
Mix cattle with others from different herds during grazing	No	90	8.89(3.92-16.77)	1(Ref)	
	Yes	499	3.61(2.15-5.64)	0.4(0.2-0.9)	0.029
Shared watering points between villages	No	253	3.95(1.91-7.15)	1(Ref)	
	Yes	336	4.76(2.75-7.62)	1.2(0.5-2.7)	0.636
Shared water sources within villages	No	27	0(0-1.28)	1(Ref)	
	Yes	562	4.62(3.04-6.71)	Infinite	0.823
Cattle contact others from adifferent herd at watering points	No	152	6.58(3.20-11.77)	1(Ref)	
	Yes	437	3.66(2.11-5.58)	0.5(0.2-1.2)	0.137
Bought livestock in the previous year	No	158	0.63(0.01-3.45)	1(Ref)	
	Yes	431	5.80(3.79-8.44)	10.1(1.3-75.8)	0.025
Bull ownership	Bull from another farm	90	12.2(6.26-20.82)	4.5(2.0-10.3)	0.0003

	Own bull	499	3.01(1.69-4.91)	1(Ref)	
Historyof abortions in the sampled herds	No	291	6.53(3.98-10.00)	1(Ref)	
	Yes	298	2.35(0.94-4.78)	0.3(0.1-10.9)	0.018

Multivariable Analyses

Table 4. 5: Results of multivariable logistic regression analysis showing risk factors found to be associated with animal-level seropositivity of Coxiella burnetii.

Variable	Category	Odds Ratio (95% CI)	P value
Fixed effects			
Zones	Zone 3	1(Ref.)	
	Zone 2	0.9 (0.2 – 4.2)	0.875
	Zone 1	0.6 (0.1 – 2.8)	0.533
Bought livestock in the previous year	No		0.190
	Yes		
Bull ownership	Own bull	1 (Ref.)	
	Use breeding bull from another farm	5.2 (1.5 – 18.4)	0.010

Ref- reference category;

CI-lower and upper limits for 95% confidence interval. Number of observations 589; number of herds 344.

The variance for the random variable (i.e., herd ID) used to account for the within–herd clustering of

Coxiella burnetii exposure was 0.00

Table 4. 6: Results of multivariable logistic regression analysis showing risk factors found to be associated with herd-level seropositivity of Coxiella burnetii.

Variable	Category	Odds Ratio (95% CI)	P value
Fixed effects			
Zones	Zone 3	I (Ref.)	
	Zone 2	1.0 (0.3-3.0)	0.949
	Zone 1	0.8 (0.2 - 2.6)	0.754
Bought livestock in the previous year	No	1 (Ref.)	
	Yes	10.8 (1.4 – 81.6)	0.021
Bull ownership	Own bull	1 (Ref.)	
	Use breeding bull from another farm	4.1 (1.7 – 10.1)	0.002
History of abortions in the sampled herds	No	1 (Ref.)	
	Yes	0.4 (0.2 – 1.0)	0.044

Ref-reference category;

CI-lower and upper limits for 95% confidence interval. Number of observations 589

Number of villages- 5.

The variance for the random variable (i.e., village ID) used to account for the within–village clustering of *C. burnetii* exposure was 0.018.

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.0 Discussion

Seroprevalence studies remain paramount when conducting prevalence studies of *C burnetii*. Through detection of antibodies the results of this study confirmed C. *burnetii* exposure to cattle in the Maasai Mara ecosystem in Narok County, Kenya. The seroprevalence of *C. burnetii* in this study was 1.87% with the rates being higher in males than females. This rate was significantly lower than those previously reported in various regions in Kenya by Nakeel et al., (2016) in Kajiado County and Knobel et al., (2013) in a study carried out in western Kenya where the prevalence rates were 89.7% and 28.3% in cattle respectively. The low prevalence reported in this study could have been due to differences in livestock production systems. The semi-extensive local production system practiced in this area maybe an attributable probable cause for the low prevalence rates because of the increased land surface area per animal (Ruiz-Fons et al., 2010) The overall seroprevalence rates of *C. burnetii* in cattle in Africa have been found to be ≤ 13% consistent with the current findings (Vanderburg et al., 2014b). The seropositivity rates in this study were higher in bulls than cows; however the reason for this remains unclear. Two studies have supported the lack of explanation on sex prevalence classifying it as a knowledge gap although the bulls being the highest shedders as compared to the cows is a putative cause (Paula Menzie; Personal communication, 2021).

The likely explanation for high prevalence rates of the disease in cattle herds grazed together mostly lie in the lack of awareness about the disease, close contact between animals during herding and when housed together and the sharing of watering points (Darryn et al., 2013). This simulates the findings of this study where communal grazing and inter-herd and inter-village grazing were seen to be greatly associated with *C. burnetii* positivity at the herd level. Increased animal density per land area could result in higher likelihoods of being directly exposed to Coxiellosis (Barlozzari et al., 2020; Dhaka et al., 2020; Vanderburg et al., 2014b).

In addition, both the current study and that of Nakeel et al., (2016), report that cases of abortions in the herd are associated with increased seropositivity to *C. burnetii*. Gravid ruminants are more susceptible to *C. burnetii* than non-gravid ruminants and this explains the reproductive failures associated with the bacteria (Khalili and Sakhaee, 2009). High rates of abortion are a public health challenge as the risk of exposure of the disease to humans is increased since it is a zoonotic disease (Guatteo et al., 2006). In a previous epidemiological study by Dean et al., (2013) among Fulani nomadic pastoralists, seropositive cattle had odds of 6.7 times more likely to have had a reproductive failure the previous year. The results of this study therefore demonstrate the need for animal confinement during parturition in addition to increased awareness on the methods of Q fever transmission so as to aid in controlling its spread. The risk variable; newly bought livestock and the correlation with high seropositivity observed in this study is in agreement with the study done on cattle in western Kenya (Wardrop et al., 2016), where farms with recently purchased cattle registered higher *C. burnetii* seropositive cases. This can be best explained by the fact that these new animals were from different herds that may have had pre-exposure to the pathogen and interherd mixing happens in market set ups. The other possible explanation is that the markets have become a pathogen hub where animals that go through this route are more likely to be exposed to a myriad of pathogens and *C. burnetii* being a pathogen that can survive in dust is a good candidate for market pathogens. Animals sent for market may travel from long distances including *C. burnetii* endemic areas or areas with predominantly higher seroprevalence and this increases the likelihood of coxiellosis in these animals. There is also the probability of farmers selling animals with reduced fertility and productivity and if the putative cause of the former is *C. burnetii* then farmers are more likely to introduce sick animals to their healthy animals once they purchase them. Based on these plausible

transmission pathways (Courcoul et al., 2011) had suggested the use of vaccination and field

side testing in the cattle markets as this could reduce the degree of shedding from the infected animals, significant reduction in the pathogen load and the number of abortions experienced due to coxiellosis (Wardrop et al., 2016).

Bull ownership also showed a spike of significance as those herds that used a borrowed bull were more likely to have an increased seroprevalence than those herds that used their own bulls. Semen has been found to be a plausible medium for *C. burnetii* and it is in line with fact that bulls that have not been tested and cleared to breed are putative causes of coxiellosis in heifers and cows (Kruszewska and Tylewska-Wierzbanowska, 1997). Cattle owners can only be sure of their own bulls and not be confident in borrowed bulls that is why there is an increased seropositivity seen when borrowed bulls are used for breeding as opposed to own farm bull.

IFAT test (Immunofluorescent assay test) is the gold standard test for the diagnosis of *C. burnetii,* however the cons of using this method are many including the lack of homogenous laboratory standards and the inability to use on large set of samples due to the intense laborious laboratory work. Complement fixation test (CFT) can also be used but it is not so much prioritised while undertaking serological studies due nonspecific output when anti complementary substances are present in the sera and in the same breath some ruminant antibodies have been seen to fix complement (Rousset et al., 2007). The use of ELISA was used in this study because of the high sensitivity and specificity observed during cattle sera testing (Dean et al., 2013).

6.0 Conclusion

The seroprevalence results indicate that indeed there was exposure of C. *burnetii* antigens to cattle in the Mara ecosystem, Narok Kenya.

Animal husbandry and management have seemingly been a valuable variable in seropositive herds and this is closely interlinked with cultural differences especially in pastoral zones like Narok where this study was done.

The sharing of grazing grounds, watering points and bull borrowing is more prevalent in such set ups as opposed to intensified husbandry systems.

The farm animal-wildlife interface zones proved to be less significant but more studies should focus on the disease drivers in such interface and remove confounders for a more conclusive grip on the risk factor.

7.0 Recommendations

- A good *C. burnetii* epidemiological picture should include other ruminants (sheep and goats) and Humans beings and report more on the seroconversions into clinical disease.

- High quality data should be available for identification and mapping of endemic areas to be able to establish concrete transmission dynamics and unexplored risk factors; thus more elaborate studies of clinical cases of Q fever should be done.

- The veterinary arm should be more vigilant in pen-side diagnostics and surveillance action models especially in markets and endemic areas. A One Health approach should be used as the supreme guide C. *burnetii* studies where both the animal and human interfaces are well captured in aim to provide comparative support for disease control and prevention strategies.

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