SERO-PREVALENCE OF COXIELLA BURNETII INFECTION IN SHEEP AND GOATS AND ASSOCIATED FACTORS IN BARINGO COUNTY, KENYA

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Sero-prevalence of *Coxiella burnetii* infection in sheep and goats and associated factors in Baringo County, Kenya

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A thesis submitted in partial fulfilment of the requirements for the award of a Master of Science degree in Applied Epidemiology of the Jomo Kenyatta University of Agriculture and Technology.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University supervisors

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DEDICATION

This thesis is dedicated to my wife, Faith Mulei - the arc of our family, our lovely daughter Terryann Mulei - my source of inspiration, our son Tehinnah Mulei – the favored one and my sister in-love Regina Mwende. Thank you for the love, support, encouragement and your invaluable prayers.

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ACRONYMS AND ABBREVIATIONS

CDC	Centres for Disease Control and Prevention
CFT	Compliment Fixation Test
CVL	Central Veterinary Laboratories
DVBD	Division of Vector Borne Disease
DVS	Director of Veterinary Services
ELISA	Enzyme-linked Immunosorbent Assay
FELTP	Field Epidemiology and Laboratory Training Program
IFA	Indirect Immunofluorescence Test
ILRI	International Livestock Research Institute
IREC	Institutional Research Ethics Committee
ITROMID	Institute of Tropical Medicine and Infectious Diseases
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KNBS	Kenya National Bureau of Statistics
MoALF	Ministry of Agriculture, Livestock & Fisheries
NDMA	National Drought Management Authority
OIE	World Organization for Animal Health
PPEs	Personal Protective Equipment's
qPCR	quantitative Polymerase Chain Reaction
WHO	World Health Organization

ZDU	Zoonotic Disease Unit
LOHs	Livestock Owning Households

DEFINITION OF TERMS

‘**Agro-pastoralism**’ refers to practice of people mainly involved in keeping of livestock as well as minor cultivation of land.

‘Herd’ refers to any aggregate of animals (sheep and goats), under the same management system but not necessarily owned by a single individual that share common risk factors for disease hence distribution of disease within the animals is relatively homogenous.

‘Household’ refers to individuals living under one roof and who share one cooking pot

‘Mixed farming system’ refers to People whose livelihoods depend equally on livestock rearing and crop farming.

‘Nomadic pastoralism’ refers to regular round-trip from home base to pasture (e.g., move herds up into national park/forest pastures in dry season, back in wet season), without any major dwellings in any location

‘Pastoralism’ refers to subsistence system based primarily on domesticated animal production (meat, milk, hides, blood).

ABSTRACT

Q fever is a highly endemic, globally distributed zoonosis caused by *Coxiella burnetii.* The pathogen host rage is varied although domestic small ruminants are the frequent source of human infection. The disease is associated with outbreaks of significant public and animal health importance but epidemiological data in sub-Saharan Africa is largely scarce with little attention to control and prevention strategies. The aim of this thesis was to determine the seroprevalence and factors associated with *C. burnetii* infection in small ruminants both at herd and animal level in Baringo County, Kenya, a region where acute cases of Q fever in humans have previously been reported. Data were collected from 140 randomly selected households. From the 140 household herds, 508 goats and 332 sheep were randomly selected for blood sample collection and the serum analyzed using an indirect ELISA assay for *C. burnetii* IgG antibodies. In addition, epidemiological information at both herd and individual animal level was collected using an animal level factor data collection tool and a household questionnaire. At individual animal level, the tool captured animal characteristics such as age, sex, species, breed, and production system while at household/herd level; the questionnaire captured household demographics, husbandry practices and farmer practices. Statistical analysis was done both at animal level and herd level. Multivariable logistic regression model was used to evaluate the relationship between *C. burnetii* seropositivity and animal- and herd level factors. A mixed effect multivariable model using household herd as a random effect was used to adjust the data for possible clustering with *C. burnetii* seropositivity. The overall small ruminant seroprevalence was 20.5%. Seroprevalence was significantly higher in goats than in sheep (26.0% versus 12.2%, p = <0.001). Of the 140 households enrolled, 92 (66% 95% CI: 57.6 -73.2) had atleast one animal seropositive. At animal level, production system (nomadic pastoralism) and animal age (old versus young) were significantly associated with positive serological result (p = <0.05). Heterogeneity in *C. burnetii* seropositivity was observed across the sub locations (p =0.028). At herd/household level, households practicing nomadic pastoralism were more likely to be *C. burnetii* seropositive compared to those practicing non-nomadic pastoralism production

systems (OR= 7.6, 95% CI: 2.3-34.1), p = 0.0023. Of the 140 humans interviewed, 120 (86%) reported to have assisted animals giving birth, 115 (82%) assisted in removal of retained placenta and 78% (n=109) had contact with aborted fetuses. Only 4 (3%) reported to have used PPEs during contact with animals. *Coxiella burnetii* exposure in sheep and goats and associated factors were demonstrated. Integrated animal-human surveillance and socio-economic studies are required for *C. burnetii*, to aid on the understanding of the risk of transmission between the animals and humans, and in the design of prevention and control strategies for the disease in the region.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Q fever is a highly endemic, globally distributed zoonosis caused by the obligate intracellular gram negative bacterium, *Coxiella burnetii* (Maurin & Raoult, 1999). Domestic small ruminants are the main reservoirs of *C. burnetii,* although its host range is varied including wild and domestic ruminants, dogs, cats, birds, arthropods and reptiles (Maurin & Raoult, 1999). Several tick species have also been identified as vectors of *C. burnetii*, as was demonstrated by detection of the pathogen in ticks (Knobel *et al*., 2013).

Transmission to humans is primarily by exposure to infected livestock especially parturient animals, their products or their contaminated environment (Maurin & Raoult, 1999). *Coxiella burnetii* infection is mainly through inhalation of aerosolized contaminated material from infectious livestock parturition fluids, vaginal mucus, milk, urine, faeces or contaminated environment (Porter, Czaplicki, Mainil, Guatteo, & Saegerman, 2011). Q fever has been classified as an occupational zoonosis especially to those exposed to ruminant animals and their products such as veterinarians, farmers, breeders, wool shearers and slaughter house workers (Van den Brom, Schimmer, *et al*., 2013).

However, outbreaks have been reported in urban areas in people with no history of contact with livestock or livestock products (Amitai *et al*., 2010) , which may be attributed to the fact that *C. burnetii* is resistant to harsh environmental conditions and can persist in the environment for months to years (Gurtler *et al*., 2014).

Airborne transmission of *C. burnetii* has been reported where the bacterium is carried by wind, causing outbreaks (Tissot-Dupont, Amadei, Nezri, & Raoult, 2004). Livestock trade and animal movement have also been shown to contribute to *C. burnetii* transmission and spread dynamics between herds (Nusinovici, Frossling, Widgren, & Beaudeau, 2015; Pandit, T. Hoch, Ezanno, & Vergu, 2016). Local

environmental conditions such as vegetation and soil moisture have also been shown to play an important role in *C. burnetii* transmission from infected farms (van der Hoek, Hunink, Vellema, & Droogers, 2011).

Evidence of transmission by indirect contact through aerosolization of the bacterium has also been documented as reported in the 2007 – 2010 Netherlands outbreaks (Roest *et al*., 2011; Schimmer *et al*., 2011). In humans, the disease presents as acute or chronic form; with the acute disease being characterized by fever, atypical pneumonia and hepatitis while the chronic form manifests with endocarditis (Anderson *et al*., 2013; Wielders *et al*., 2014). Q fever associated mortality is usually low (1 – 2%) in treated patients (Tissot Dupont *et al*., 1992) but the mortality may be as high as 65% in infected persons who develop chronic disease (Raoult, Marrie, & Mege, 2005).

In domestic ruminants, the disease is characterized by reproductive disorders including abortions, stillbirths, premature delivery and birth of weak offspring's (Agerholm, 2013). Domestic small ruminants pose public health risk of Q fever outbreaks as was documented in the 2005 Germany outbreak where 331 human cases were associated with sheep grazing and lambing near a residential area (Gilsdorf, Kroh, Grimm, Jensen, & C., 2008) and in the Netherlands outbreak between 2007 – 2010 where over 4000 human cases were associated with exposure to goat herds (Delsing, Kullberg, & Bleeker-Rovers, 2010).

Q fever has a worldwide distribution with the exception of New Zealand (Hilbink, Penrose, Kovacova, & Kazar, 1993). However, most of the Q fever risk analysis studies have been conducted in the developed countries in Europe where the livestock rearing systems are different from the ones practiced in resource poor settings in sub-Saharan Africa (Georgiev *et al*., 2013). The Netherlands outbreak has been associated with the increased goat population in the country, which emphasizes the public health risks of Q fever epidemics posed by domestic ruminants (Roest *et al*., 2011). In spite of the notable nature of some of the Q fever outbreaks, and the global health security risk *C. burnetii* poses as a potential bioterrorism agent (Madariaga, Rezai, Trenholme, & Weinstein, 2003), data on the epidemiology of *C.*

burnetii infection in sub-Saharan Africa is largely unavailable (Vanderburg *et al*., 2014).

In Kenya, evidence of *C. burnetii* exposure has been reported since 1950s, where the prevalence of *C. burnetii* antibodies in humans was reported to range from 10% to 20% (Njeru, Henning, Pletz, Heller, & Neubauer, 2016). In an investigation of a group of 50 travellers for a safari trip to Kenya, four people (8%) were found to have contracted Q fever (Potasman, Rzotkiewicz, Pick, & Keysary, 2000). Among domestic ruminants in Kenya, previous studies have documented the prevalence of *C. burnetii* antibodies to be 7 – 57% in cattle and 33 – 34 % in goats (Njeru *et al*., 2016).

In rural western Kenya, *C. burnetii* antibodies were detected in 30.9% of humans, 28.3% of cattle, 32.0% of goats and 18.2% of sheep (Knobel *et al*., 2013). A study in Laikipia County, Kenya, showed a distinct seroprevalence gradient, ranging from 0% to 4% in cattle, 13% to 20% in sheep, 31% to 40% in goats and 5% to 46% in camels (DePuy *et al*., 2014). A recent linked human-cattle population survey in western Kenya detected prevalence of 2.5% in humans and 10.5% in cattle (Wardrop *et al*., 2016).

In 2014, cases of acute Q-fever in humans were reported in Baringo County with 54.8% of human samples collected during an outbreak investigation being positive for anti-phase II antibodies, and confirmed through quantitative polymerase chain reaction (qPCR) [Baringo County Health department, unpublished data]. In Baringo County Kenya, the main livestock rearing systems practiced are mainly nomadic pastoralism and agro-pastoralism characterized by free roaming movements, common grazing, watering points and live animal markets which pose challenge in applying necessary level of biosecurity (Beneah, 2014).

These factors contribute to increased frequency of livestock contact which in turn contributes to disease transmission and spread between herds. This system being different from the livestock production system in Europe where most *C. burnetii* risk analysis studies have been conducted highlights the importance of *C. burnetii*

epidemiological studies in these less studied systems, which might provide new and important insights on Q fever epidemiology including intra and inter herd transmission dynamics.

The study aimed at determining the seroprevalence of *C. burnetii* in goats and sheep from the same region where the outbreak had been reported, and investigating the factors associated with *C. burnetii* infection in Baringo County, Kenya.

1.2 Statement of the Problem

Q fever is a common zoonotic disease with worldwide occurrence except in New Zealand (Hilbink *et al*., 1993). *Coxiella burnetii* is currently considered a potential agent of bioterrorism and is classified by the Centers for Disease Control and Prevention as a group B biological agent meaning it's easy to disseminate, has moderate morbidity rates, low mortality rates and requires specific enhancements in diagnostic capacity and enhanced disease surveillance (Glazunova *et al*., 2005). The disease can also have a negative impact on global travel and trade (Ta *et al*., 2008).

Q fever infection in sheep and goats causes heavy economic losses in terms of disease control costs, culling infected animals, breeding prohibition, abortions and birth of weak offspring (Maurin & Raoult, 1999). The disease in humans is of great public health implication mainly due to the long term sequelae (R. J. Brooke, Van Lier, Donker, Van Der Hoek, & Kretzschmar, 2014), it accounts for a big proportion of non-specific human febrile illness as well as various diseases such as acute flu-like illness, pneumonia, hepatitis and chronic endocarditis (Marrie, 2003).

Despite Q fever being reported worldwide both in humans and domestic small ruminants, there is no published data on the burden of Q-fever in sheep and goats in Baringo County, although the disease has been suspected on basis of clinical presentation. Humans mainly acquire the infection from infected animals through inhalation of infected aerosols due to close interaction with domestic ruminants a factor that puts residents of Baringo County at high risk for infection since more than 80% of the residents derive their livelihoods from livestock especially sheep and goats.

Evidence of exposure to *C. burnetii* in humans from Baringo County has been reported during surveillance for acute febrile illnesses by Walter Reed Project (Walter Reed Project unpublished data). This makes the case for a study to identify the burden of Q-fever and associated factors in domestic small ruminants even more strong so as to provide a more complete picture of the disease epidemiology, understanding the disease risk and providing data for instituting effective control and prevention measures.

1.3 Justification of the Study

Livestock plays a crucial role in the livelihood of the majority of residents of Baringo County. Majority are predominantly pastoralists keeping mainly sheep, goats cattle and camels which provide source of livelihood to over 80% of the residents. Small ruminants (sheep and goats) are ranked high in importance as a source of regular cash income and as insurance against emergencies. However this dependence on livestock increases the vulnerability of the people to zoonotic diseases. Some of the socio-cultural practices such as consumption of raw unpasteurized milk and close interactions with animals including assisting during parturition make residents of Baringo County at risk of exposure and infection with Q fever.

In 2014, an outbreak investigation in the County following reports of deaths of six people, report of laboratory findings showed that 6 (19.4%) and 12 (38.7%) of 31 human samples were positive by quantitative polymerase chain reaction (qPCR) and anti-phase II antibodies, respectively, for *C. burnetii*. In total, there was evidence of acute Q-fever infection in 17 of 31 (54.8%) (Walter Reed Project unpublished data).

Incidence of sheep and goats abortion was high, mainly in late gestation without specific clinical signs. Although brucellosis and other causal agents may be responsible for a considerable percentage of abortions in Baringo County, there remain a significant number of abortions with unknown etiology each year. Although there are no published studies that incriminate *C. burnetii* as a plausible cause of abortions in sheep and goats in Baringo County, Q fever has been suspected on basis of clinical presentation. However, reliable data on Q fever in Kenya is scarce and

data on prevalence and factors associated with sheep and goat Q fever in Baringo County is not known.

Data from this study will be useful in determining the risk of Q fever in the County and provide baseline data for a possible study of Q fever in humans living in the area. Available options for control and prevention of Q fever will be explored and appropriate recommendations made.

1.4 Research Questions

1. What is the seroprevalence of *Coxiella burnetii* infection in sheep and goats in Baringo County?
2. What are the animal-level factors associated with *Coxiella burnetii* infection in sheep and goats in Baringo County?
3. What are the livestock management practices associated with *Coxiella burnetii* infection in sheep and goats in Baringo County?
4. What are the household-members' practices that are risky for zoonoses exposure in Baringo County?

1.5 General objective

To determine the sero-prevalence and factors associated with *Coxiella burnetii* infection in sheep and goats in Baringo County.

1. 6 Specific objectives

1. To determine sero-prevalence of *Coxiella burnetii* infection in sheep and goats in Baringo County.
2. To determine animal-level factors associated with *Coxiella burnetii* infection in sheep and goats in Baringo County.
3. To determine livestock management practices associated with *Coxiella burnetii* infection in sheep and goats in Baringo County.
4. To identify household-members' practices that are risky for zoonoses exposure in Baringo County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Q fever

Q fever remains an important and prevalent public health problem, but the true burden remains unrecognized in most countries because of poor surveillance of the disease (Maurin and Raoult, 1999). The disease is mainly an occupational hazard and is mostly associated with persons in close contact with domestic animals such as farmers, veterinarians, abattoir workers and laboratory personnel performing *C. burnetii* culture or working with *C. burnetii* infected animals (Anderson *et al*., 2013; Chang *et al*., 2010). However, sporadic cases and outbreaks in people living in urban settings after contact with infected pets or fomites have been reported (Amitai *et al*., 2010). Q fever has also been reported in people without history of exposure or contact with infected reservoirs hence wind may also play a part in *C. burnetii* transmission (Domingo *et al*., 1999).

Coxiella burnetii infection in humans in most cases is asymptomatic and may recover without hospitalization. However, sometimes Q fever may lead to fatal complications especially in patients with acute disease with complications such as meningoencephalitis and myocarditis, or people with chronic Q fever with endocarditis. People with history of cardiac valve defects, immunocompromised and pregnant women are at risk of developing chronic Q fever. Abortion, premature birth, and underweight babies have been reported in pregnant women suffering from Q fever (Porter *et al*., 2011).

The clinical manifestations of Q fever vary widely and the definite diagnosis of the disease is based on serology, with phase I and phase II antibodies distinguishing acute from chronic disease where phase II antibodies are positive in acute disease and phase1 antibodies are elevated in chronic disease.

2.2 The Causative Agent of Q fever

Coxiella burnetii which is the causative agent of Q fever is an obligate intracellular gram-negative bacterium measuring approximately 0.2 – 1.0μm. *Coxiella burnetii* has very high infectivity in humans with a single viable *C. burnetii* being able to cause infection in humans (Russell John Brooke, Kretzschmar, Mutters, & Teunis, 2013). *Coxiella burnetii* displays different morphological forms in its developmental cycle with three forms identified namely; small cell variant, large cell variant and spore like particles.

The bacterium exhibits characteristic antigenic variation where it can undergo sporulation making it resistant to adverse environmental conditions making it easy to disseminate (Ta *et al*., 2008). *C. burnetii* exhibits phase variation with two phases I and II which differ in terms of virulence, where by phase I is very contagious and infectious with between 1 – 10 *C. burnetii* being able to cause infection in humans while phase II shows low virulence levels (Gurtler *et al*., 2014). *Coxiella burnetii* has been isolated from blood, lungs, spleen, liver, urine, feces, milk and birth products of infected humans and animals.

2.3 Epidemiology of Q fever

2.3.1 Geographical distribution of Q fever

Q fever is reported worldwide except in New Zealand (Hilbink *et al*., 1993). However in most countries, the disease is not among the list of notifiable diseases which limits the importance accorded to the disease in terms of surveillance and research, consequently the disease is classified as a neglected zoonotic disease (Anderson *et al*., 2013). Despite *C. burnetii* being classified as a category B pathogen and a potential biological weapon by the Centre of Disease Control and Prevention and the reported large scale outbreaks in the recent past, epidemiological data is scanty especially in Africa where most of the available data is only from outbreak investigations, serological surveys in humans and livestock, and laboratory records (Vanderburg *et al*., 2014).

2.3.2 Reservoirs of *Coxiella burnetii*

Several *C. burnetii* reservoirs have been documented including wild animals, domestic animals, birds, and arthropods mainly ticks. However, in most published studies, domestic ruminants are reported to be the most incriminate source of C. *burnetii* infection in humans. The disease in animals is mostly asymptomatic and in most cases proceeds to chronic phase. In female animals, uterus and mammary glands are the primary sites of chronic *C. burnetii* infection. Environmental contamination with *C. burnetii* occurs mainly during parturition when a lot of *C. burnetii* pathogens are shed to the environment. *C. burnetii* has also been isolated from milk samples hence milk may also be a source of *C. burnetii* infection in humans (Maurin & Raoult, 1999).

Coxiella burnetii has been isolated from birds such as pigeons, chickens, ducks, geese, and turkeys and hence infected poultry and poultry products may be a source of infection to humans. Pets such as cats and dogs have also been demonstrated to be reservoirs of *C. burnetii* and are becoming an important source of sporadic cases and outbreaks of Q fever in urban areas. Infection in dogs has been reported through bites by infected ticks, consumption of placentas or milk from infected ruminants, and inhalation of infected aerosols (Maurin & Raoult, 1999).

2.3.3 Mode of Transmission of *Coxiella burnetii*

Inhalation of infected aerosols from animal reservoirs is widely reported to be the main mode of transmission from infected animals to humans (Porter *et al*., 2011). *Coxiella burnetii* infected aerosols may be transmitted by direct contact but also sometimes wind/ aerosolization is reported to play a part in transmission (Ave, 2007). *Coxiella burnetii* is resistant to environmental conditions and remains in the environment after contamination for a long time which explains the occurrence of *C. burnetii* infection in patients without history of animal contact. *Coxiella burnetii* has been isolated in ticks and the role ticks play as putative vectors and reservoirs for *C. burnetii* in animals has been documented (Fard & Khalili, 2011).

2.4 Pathogenesis of Q fever

Inhalation of infected aerosols is the main route of acquiring *C. burnetii* infection in humans, however the disease has also been reported to occur through ingestion route mainly by consumption of contaminated dairy products although this is rare . The incubation period of acute Q fever range from 1 to 3 weeks, although this depends on the *C. burnetii* dose. *Coxiella burnetii* infection in most cases is asymptomatic or manifests as a non-specific flu-like illness; a factor that makes the disease mostly undiagnosed and under-reported (Raoult *et al*., 2005).

The disease has two phases of clinical presentations - acute and chronic. The acute phase mainly presents with atypical pneumonia and hepatitis and the presence of *C. burnetii* may be demonstrated in the blood, lungs, spleen, and liver. The chronic phase is mostly manifested by endocarditis. However, animals do not develop chronic endocarditis as is observed in humans. In animals chronic *C. burnetii* infection is associated with abortions, mainly in sheep and goats, and birth of underweight calves and infertility in cattle (Maurin & Raoult, 1999).

2.5 Clinical Presentation of Q fever

2.5.1 Clinical Presentation in Humans

Coxiella burnetii infection may present with acute or chronic clinical manifestations. However, majority of Q fever cases are asymptomatic and recover without need for hospitalization although a small percentage may require hospitalization. Symptomatic acute Q fever may occur and presents mainly with a self-limiting febrile illness, atypical pneumonia, or hepatitis, with endocarditis being a more common presentation of chronic Q fever (Anderson *et al*., 2013).

In symptomatic patients, the documented signs include fever, fatigue, chills, headaches, myalgia, sweats, cough, nausea, vomiting, chest pain, diarrhea, and skin rash (Anderson *et al*., 2013).

In chronic Q fever infection, endocarditis is the major clinical presentation and it accounts for 60 to 70% of all chronic Q fever cases. Mortality from Q fever is low

mostly less than 10% especially when proper antibiotic treatment is done (Porter *et al.*, 2011).

2.5.2 Clinical Presentation in Animals

Animals are often chronically infected but do not experience symptoms of *C. burnetii* infection. The uterus and mammary glands of female animals are the sites of chronic *C. burnetii* infection (Maurin & Raoult, 1999). In domestic ruminants, the disease is mainly asymptomatic but clinical manifestation through reproductive disorders such as abortions, still births, infertility and birth of weak offspring has been reported (Agerholm, 2013; Asadi, Kafi, & Khalili, 2013).

The disease causes heavy economic losses to flocks due to abortions and birth of weak offspring (Eibach *et al.*, 2012). However in other cases, animals recover without complications. *Coxiella burnetii* infection may persist for several years, and domestic ruminants are mainly subclinical carriers, but can shed bacteria in various excretions such as urine, milk, feces, placental and birth fluids (Abbasi, Farzan, & Momtaz, 2011).

2.6 Diagnosis of Q fever

2.6.1 Collection and Handling of Clinical Specimens

Coxiella burnetii is a very infectious agent, and cases of Q fever acquired from laboratory work have been described (Gurtler *et al.*, 2014). Thus, clinical materials from patients supposedly infected with C. burnetii or working with C. burnetii infected animals should be handled with care by experienced personnel wearing gloves and masks and can only be done in biosafety level 3 laboratories (Maurin & Raoult, 1999).

2.6.2 Identification of Q fever Causative Agent

There are various methods available for demonstration of *C. burnetii* although the method of use depends mainly on the type of sample and the purpose of investigation. Q fever is considered an under-reported and under-diagnosed illness mainly due to the fact that the symptoms are mostly non-specific, making diagnosis difficult (Anderson *et al*., 2013).

2.6.3 Isolation of *Coxiella burnetii*

Culture of *C. burnetii* is not recommended for routine diagnosis since it is difficult, time consuming, and can only be done in biosafety level 3 laboratories. Isolation of *C. burnetii is* mainly by culture from blood, milk, liver biopsy and fetal specimens after abortion although it's done in very few laboratories due to high risk of transmission to laboratory workers and also the fact that the technique has low sensitivity (Maurin & Raoult, 1999).. Isolation of *C. burnetii* in patients with chronic Q fever is complicated by antibiotics administration, and hence a negative culture does not necessarily mean absence of C. *burnetii* infection (Anderson *et al*., 2013).

2.6.4 Serological Methods of Q fever Diagnosis

Q fever diagnosis is mainly by serological methods such as; indirect immunofluorescence (IFA) test, enzyme-linked immunosorbent assay (ELISA), and complement fixation test (CFT) since culture and molecular techniques have low sensitivity and have limited availability (Maurin & Raoult, 1999).

Serological diagnosis is easy to perform and allows the differentiation of acute and chronic Q fever infections through testing both acute and convalescent sera. The presence of specific IgG antibodies provides evidence of a recent *C. burnetii* infection or a past exposure depending on the antibody titres and phase variation. Enzyme -linked immunosorbent assays s are preferred for practical reasons such as ability to test large number of sera and also high sensitivity (EFSA Panel on Animal Health and Welfare (AHAW), 2010).

a) Indirect Immunofluorescence Assay (IFA)

Indirect Immunofluorescence Assay involves use of phase I and phase II *C. burnetii* antigens to differentiate between acute and chronic Q fever depending on IgG and IgM antibody titers against Phase1 and Phase11 antigens. Different serological profiles are observed in acute and chronic forms of infection whereby, during acute Q fever, IgG antibodies are elevated against phase II only whereas during chronic Q fever, high levels of IgG antibodies to both phase I and II of the *C. burnetii* are observed.

An increase in phase II IgG antibody titer by IFA of paired acute and convalescent sera by fourfold is considered the gold standard diagnosis to confirm acute Q fever (Anderson *et al*., 2013).

b) Complement Fixation Test (CFT)

Complement fixation test involves detecting complement-fixing antibodies presence in serum. The reaction is done in two stages where, in the first stage, antigen and complement-fixing antibodies are mixed, and sheep erythrocytes, sensitized by the anti-sheep erythrocyte serum are then added (Maurin & Raoult, 1999). Complement fixation by the antigen/antibody complex in the first step does not allow lysis of erythrocytes, but if there is no complex formed the free complement induces lysis of the sensitized erythrocytes thus hemolysis denotes a negative reaction (Maurin & Raoult, 1999).

c) Enzyme-linked Immunosorbent Assay (ELISA)

Enzyme – linked Immunosorbent Assay is routinely used due to its high sensitivity, good specificity as well as it is relatively easy to perform in laboratories with appropriate equipment and reagents. Enzyme-linked immunosorbent assay is preferred to IFA and CFT, especially for demonstrating *C. burnetii* in animals due to its ability to handle large quantity of samples and can test multiple species. Commercial kits are readily available and can detect both anti-phase I and II antibodies (Anderson *et al*., 2013).

2.6.5 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction involves detection of *C. burnetii* DNA and can be used to diagnose acute Q fever especially within the first two weeks after onset of symptoms but before administration of antibiotics. Polymerase Chain Reaction can be performed on multiple specimen types such as whole blood, serum and tissues. The technique has high sensitivity and rapid results turn-around (Maurin & Raoult, 1999).

2.7 Treatment of Q fever

Most of the acute Q fever cases resolve spontaneously even without treatment. However confirmed or suspected cases should be treated with doxycycline which is the most effective treatment for Q fever. It’s advisable for treatment to be given within the first 3 days of onset of symptoms since this shortens the illness and reduces the risk for developing severe complications (Anderson *et al*., 2013).

2.8 Control and Prevention of Q fever

Q fever in humans is mainly an occupational hazard involving people who are in frequent contact with infected animals and animal products. Most of the groups at risk include; veterinarians, livestock farmers, and researchers manipulating *C. burnetii* or working with *C. burnetii* infected animals. Prevention and control efforts should be directed towards controlling the infection at the reservoir host, risk groups and the environment. Measures for prevention and control of Q fever include;

- Public awareness creation on sources of Q fever infection.
- Appropriate disposal of placenta, after births, fetal membranes, and aborted fetuses at facilities housing domestic ruminants.
- Restrict access to barns and laboratories used in housing potentially infected animals.
- Avoid use of raw milk and milk products.
- Vaccinate (where possible) individuals engaged in research with infected animals or live *C. burnetii*.

- Quarantine imported animals.
- Surveillance for early detection of cases/antibodies to *C. burnetii*, and strict implementation of control measures (Anderson *et al*., 2013).

Q fever vaccine has been developed and has successfully protected humans in Australia who have been occupationally exposed. However, this vaccine is not widely available in many other countries. A vaccine for use in animals has also been developed, but it is also not widely available (Delsing *et al*., 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Baringo County is located in the former Rift valley province of Kenya and borders Turkana and Samburu counties to the north, Laikipia to the east, Nakuru and Kericho to the south, Uasin Gishu to the southwest, and Elgeyo-Marakwet and West pokot to the west **(Figure 3.1)**. The county covers an area of 11,015.3 square kilometers with an estimated population of 555,561 (Kenya National Bureau of Statistics, 2010). The County includes 165 km^2 of Lake Baringo and Lake Bogoria. It has a bimodal rainfall pattern with the long rains falling in March to May and short rains from August to November. Temperatures range from 10^0 C in the highlands to 37^0 C in the lowlands with the hottest months being January to early March (NDMA, 2014).

The County is divided into four livelihoods zones namely; mixed farming, Pastoral, Agro-pastoral and irrigated agriculture. The proportion of the population in each livelihood is 43, 31, 22, and 4 percent respectively. The county has an altitude ranging from 800 meters in the lowlands to 300 meters in the highlands and receives an annual rainfall of 500 mm in the lowlands and up to 1,500 mm in the highlands. Administratively the county is divided into six sub-counties namely; Mogotio, Baringo North, Baringo central, East Pokot, Koibatek and Marigat. The main livestock species kept within the county include cattle, goats, sheep, camels and donkeys. Others include poultry and bee keeping. Livestock contribute 88, 50, 23, and 5 percent of income in pastoral, agro-pastoral, mixed farming and irrigated farming livelihood zones respectively (NDMA, 2014).

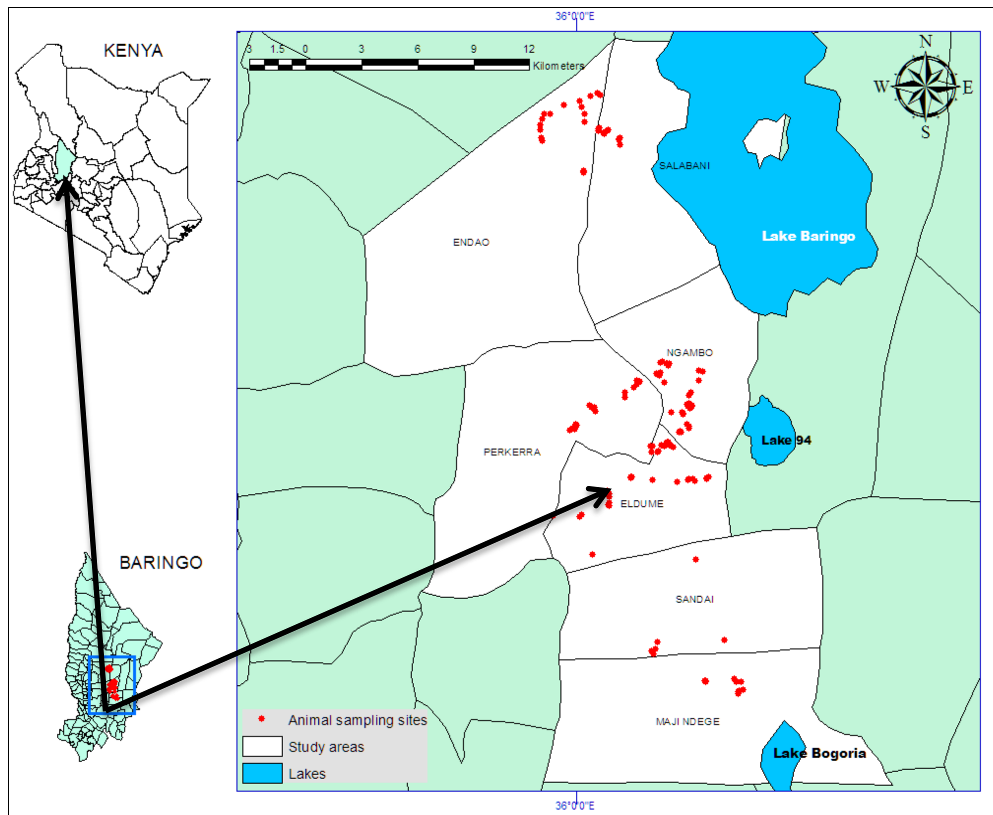

Figure 3. 1: Map of study area in Baringo County, Kenya

3.2 Study Design

This was a cross-sectional study conducted in Baringo County.

3.3 Study Population

Goat and sheep populations in livestock owning households (LOHs) and household heads or their representatives in the selected LOHs in the study area will comprise the study population. According to 2009 Kenya national housing and population census, the sheep and goat population in the county was estimated as shown (Table 3.1)

Table 3. 1: Sheep and goats population, Baringo County

District	Sheep	Goats
Baringo Central	72,260	168,852
North Baringo	30,446	128,364
East Pokot	380,000	1,474,617
Total	**482,706**	**1,771,833**

Source: Kenya National Bureau of Statistics, 2009 population and housing census report.

3.4 Sample Size Determination

Households with small ruminant herds/flocks were the primary sampling units while the individual animals within the herds/flocks were the secondary sampling units. A herd/flock was defined as any aggregate of animals (sheep and goats), under the same management system but not necessarily owned by a single individual that share common risk factors for disease hence distribution of disease within the herd is relatively homogenous (Thrusfield, 2007). Sample size calculation was based on the formula for sample size determination when herds, flocks or other aggregates of animals are the sampling units and taking into account herd effects to achieve high herd level sensitivity and specificity while also accounting for test imperfections since the ELISA kit used had less than 100% sensitivity and specificity (Humphry, Cameron, & Gunn, 2004; Thrusfield, 2007).

$$n = (1.96/d)^2 \text{ X } \frac{[(Se_{\text{agg}} \text{ x } P_{exp}) + (1 - Sp_{\text{agg}})(1 - P_{exp})][(1 - Se_{\text{agg}} \text{ x } P_{exp}) - (1 - Sp_{\text{agg}})(1 - P_{exp})]}{(Se_{\text{agg}} + Sp_{\text{agg}} - 1)^2}$$

Assumptions made:

n = required number of herds or flocks to be sampled

P_{exp} = expected herd or flock prevalence (50%) - 50% herd/flock prevalence was assumed since it provides the largest sample size for given values of absolute error.

d = desired absolute precision (±10%)

Se_{agg} = Aggregate test sensitivity determined by investigator (97%)

Sp_{agg} = Aggregate test specificity determined by investigator (94%)

Based on the above assumptions **140 household herds** were included in the study.

The number of herds was distributed to the sub locations proportionate to number of herds in the sub-location (Sampling proportion to size). From each herd, a maximum of six (6) animals were selected making the total number of animals (sheep and goats) sampled to be 140 X 6 = **840 animals** (508 goats and 332 sheep).

3.5 Sampling Design

This was multi-stage sampling process where the first stage involved selection of sub-locations to be included in the study, followed by selection of sheep and goat herds/ flocks and finally individual animals. In each stage simple random sampling technique was used to select the units of interest(Thrusfield, 2007). In this case households with small ruminant herds/flocks were the primary sampling units while the individual animals within the herds/flocks were the secondary sampling units. From the two sub-counties, a list of all the sub-locations as per the 2009 KNBS census was drawn and 10% (n=7) of the total sub locations were randomly selected and included in the study.

The choice of 10% of the sub locations was mainly due to limited financial resources. To select the sub locations, small folded papers with names of the sub locations was put in a basin and a blindfolded person was asked to pick randomly until the required number was attained. An updated list of sheep and goats herds in

the selected sub-locations was generated in consultation with local administrators, animal health staff and community elders in a baseline survey taking into account herd definition mentioned earlier and used as the sampling frame for actual sampling. From the sampling frame of 250 in the seven sub locations, 140 Livestock owning households were randomly selected using computer generated random number format.

Due to logistical constrains a maximum of six (6) animals (sheep and goats) of any age were randomly selected from the herd for blood sample collection. Individual animals were selected using the lottery method were all animals in the herd were numbered using animal marker pens and random numbers assigned by dividing the herd size by six (number of animals selected per herd)to create the interval of selection. Animals bearing the random number were selected for blood sample collection. In this system, sheep and goats are managed together as one herd hence the above sample size applied to both sheep and goats.

3.6 Blood Sample Collection and Serological Analysis

Blood samples were collected by jugular venipuncture as per the standard operating procedures for animal experimentation (International Livestock Research Institute, 2004). A volume of 5 mls of blood was collected into vacutainers, and transported in a cool box to the field laboratory (Marigat DVBD laboratory). Serum was harvested by centrifugation at 2500g for 10 minutes, serum collected into cryovials and transported to the Central Veterinary Laboratory, Kabete where it was stored at -20° C until testing.

Anti - *C. burnetii* IgG antibodies were detected using a commercially available indirect ELISA kit (LSIVet^{TM} Ruminant Q fever – ELISACOXLS2) following manufacturer's instructions. Briefly, 5µl of pre-diluted serum samples and controls (positive and negative) were added in the wells of the coated plate and 95 µl of Q fever sample dilution buffer added to each well containing the controls or samples. The plate(s) was then covered with an adhesive cover plate and incubated for one hour at 37^{0}C.

Three washes were performed with wash solution before 100 µl of conjugate were added and incubated for one hour at 37^0C. The three wash steps were repeated and 100 µl of substrate added to each well and incubated for ten minutes at room temperature, then 100 µl of stop solution was added. The results were read within 30 minutes after stopping the reaction at 450 nm on a microplate reader (*Biokit, ELX800* ™ - *USA*).

The results were expressed as S/P (sample/positive) ratio calculated as S/P = (OD sample – ODm NC)/ (ODm PC – ODm NC). The percent positivity (PP) was expressed as PP = S/P x 100. Where OD sample is the optical density for the sample, ODmPC is the average optical density for the positive control and ODmNC is the average optical density for the negative control. A serum sample was considered seropositive when the PP value was greater than 40.

3.7 Questionnaire Survey

Animal, herd/flock epidemiological data, and farmers' practices risk for zoonoses exposure information and awareness on zoonoses were collected using household questionnaires **(appendix 3)** and animal sampling forms administered to the household head or his/her representative in interviews immediately after animal sample collection.

3.8 Data Management and Analysis

Data was entered into Epi info software, cleaned, validated and coded. It was checked for any wrong entry, double entry, missing data and corrected. Back up was created in case of damage and or loss of original data and stored in a password protected computer. Data was analyzed using EPI Info 7, Ms Excel 2007 and R statistical software. Descriptive analysis (means, medians and proportions) were calculated for categorical and continuous variables.

Univariable analysis was performed to determine single factor/ variable relationship with *C. burnetii* sero-positivity at herd and animal level. Bivariate analysis was carried out to evaluate the association between herd *C. burnetii* sero-positivity and

the potential risk factors. Odds ratios and 95% CI were used and factors with p-value of less or equal to 0.05 were considered significant. Multivariable analysis – Multiple Logistic regression was then used to control for known and unforeseen confounders.

Model reduction techniques from maximum to minimum adequate model that has risk factors associated with the outcome variable (*C. burnetii* sero- positivity) with factors at bivariate analysis with p-value less or equal to 0.2 considered for entry into the model was used. The model was developed by backward elimination, dropping the least significant independent variable until all the remaining predictor variables are significant (p-value less or equal to 0.05). Forward selection whereby each factor was introduced back at a time was carried out to ensure completeness.

The final fitted individual models were evaluated by including herd as a random effect to adjust for possible clustering of *C. burnetii* seropositivity. Intra-cluster correlation coefficient (ρ) describing the degree of similarity among seropositive animals in each household was estimated. Only those factors that remained statistically significant in the final model are presented.

3.9 Ethical Approvals and Considerations

Protocol approval was sought and obtained from Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT), while ethical clearance for the study was sought and obtained from the ILRI Institutional Research Ethics Committee (IREC). Written informed consent was obtained from all animal owners before specimen collection. The aim and procedures of the study were explained to the participants who were required to give written consent prior to their voluntary participation in the study.

Blood samples were collected from animals of consenting individuals. The collected serum samples were only used to detect antibodies against *C. burnetii*. Confidentiality was observed and maintained whereby all the data was anonymized through assigning all households with unique identifiers which were recorded in all forms used to collect data from the household (household questionnaire and the animal factor data collection and sample tracking tool).

The unique IDS linked the data for farm level, individual animals sampled and lab test results and any data sets shared only contained the unique IDs (no names & contacts). For confidentiality all research data was stored in locked file cabinets of study personnel offices and electronic data was stored in password protected computers. The results were shared with the county veterinary and health authorities, community and other stakeholders who are involved in surveillance in the study site. Biosafety mitigation measures were advised to the people. The results were also published in a peer reviewed journal for wide stakeholder dissemination and feedback. In Kenya there is no registered vaccine for Q fever in animals and the data generated from this study can be used to justify need for a Q fever vaccine in the country.

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics of the Sampled Animals

A total of 508 goat and 332 sheep samples were obtained from the 140 livestock owning households(LOHs) Of the animals sampled, 86% (n=723) were of indigenous breed(Red Maasai sheep and Small East African goat) and 80% (n=680) were female (Table 4.1)

Table 4. 1: Demographic characteristics of the sampled animals

Variable	Total sampled n (%)	Number positive n (%)
Species		
Goats	508(60.5)	132(25.9)
Sheep	332(39.5)	40(12.1)
Breed		
Indigenous	723(86.1)	158(21.9)
Cross-breed	117(13.9)	14(12.0)
Sex		
Female	680(80.9)	150(22.1)
Male	160(19.1)	22(13.8)
Age group		
≤1 year	168(20)	18(10.7)
> 1 - ≤2 years	236(28)	49(20.8)
> 2 - ≤3 years	197(23.5)	39(19.8)
> 3 - ≤4 years	119(14.2)	24(20.2)
> 4 years	120(14.3)	42(35.0)
Production system		
Agro pastoralism	303(41.8)	42(13.9)
Mixed farming	351(36.1)	59(16.8)
Nomadic Pastoralism	186(22.1)	71(38.2)
Sub location		
Eldume	102 (12.2)	11(10.8)
Endao	133 (15.8)	15(11.3)
Maji Ndege	60(7.1)	11(18.3)
Ngambo	125(14.9)	17(13.6)
Perkerra	72(8.6)	16(22.2)
Salabani	186(22.1)	71(38.2)
Sandai	162(19.3)	31(19.1)

Indigenous breed = locally kept breeds such as Small East African goat and Red Maasai sheep. Cross breed = indigenous breed improved by crossbreeding with exotic breeds.

4.2 Socio-Demographic Characteristics of Respondents

The survey was conducted in 140 households. Majority of the respondents, 27% (n = 38) were aged between 31 – 40 years with 60% (n=85) being male. Of all the respondents, 35% (n=50) had no formal education with only 9% (n=13) having

completed tertiary education. In terms of economic status, the household estimated monthly income for 45% and 48% of the households was KES less than10, 000 and KES10, 000 – 20,000 respectively. About 79% of the households had a semi-permanent house with only 2% owning a permanent house (Table 4.2).

Table 4. 2: Socio-Demographic Characteristics of respondents interviewed

Variable	No. of Respondents n (%)
Age group	
<31 Yrs.	30(21.4)
31 - 40 yrs.	38(27.1)
41 - 50 yrs.	22(15.7)
51 - 60 yrs.	23(16.5)
61+ yrs.	27(19.3)
Sex	
Male	85(60.7)
Female	55(39.3)
Education	
No formal education	50(35.7)
Primary	50(35.7)
Secondary	27(19.3)
Tertiary	13(9.3)
Estimated monthly income (KES)	
<10,000	63(45)
10,000 – 20,000	68(48.6)
>20,000	9(6.4)
Housing type	
Temporary	26(18.6)
Semi-permanent	111(79.3)
Permanent	3(2.1)

4.3 Household Livestock Management Demographic Characteristics

Introduction of a new animal in the herd for the past six months was reported in 15% (n=21) of the households. Herd contact during grazing was reported in 98.4% (n=138) with other herds, and 95% (n=132) reported contact with wild animals (gazelles and dikdiks) in the past six months. Tick control was reported in majority of the households, 97% (n=136) with the frequency ranging from weekly to quarterly, although majority 51.4% (n=70) did tick control after every two weeks. Breeding system was mainly natural with 84% (n=106) and 65% (n=87) reporting their bucks and rams to have bred does and ewes from other herds in the past six months. Reproductive disorders among small ruminant herds in the last six months were reported where; 67% (n=94) abortions, birth of weak young ones in 43.57% (n=61), retained placenta 11.4% (n=16), still births 4.3% (n=6) and infertility 2.8% (n=4) households reported. The livestock production systems practiced was nomadic pastoralism in 22.14% (n=31), Agro-pastoralism 41.43% (n=58), and mixed farming 36.43% (n=51) of the households (Table 4.3).

Table 4. 3: Household Livestock Management Demographic Characteristics

Variable	No. of Respondents n (%)
Introduced a new animal	21(15)
Contact with other herds	138(98.4)
Contact with wild animals	132(95)
Tick control	136(97)
Tick control frequency	
Weekly	17(12.5)
After 2 weeks	70(51.5)
Monthly	32(23.5)
Quarterly	17(12.5)
Breeding	
Bucks bred other herd does	106(84)
Rams bred other flock ewes	87(65)
Reproductive disorders	
Abortions	94(67)
Still births	6(4.3)
Weak young's	61(43.6)
Infertility	4(2.8)
Retained placenta	16(11.4)
Herd production system	
Mixed farming	51(22.2)
Agro-pastoralism	58(41.4)
Nomadic pastoralism	31(36.4)

Multiple responses allowed hence proportion calculated per response

4.4 Seroprevalence and Animal Level Factors Associated with *C. burnetii* Infection

The combined sheep and goat overall seropositivity to *C. burnetii* antibodies was 20.5% (95% CI: 17.8, 23.3), whereas it was 26.0% (95% CI: 22.2, 30.0) in goats and 12.2% (95% CI: 8.7, 16.0) in sheep. On univariable analysis, age group, breed, sex, species and nomadic pastoralism were significantly associated with *C. burnetii* seropositivity (Table 4.4).

Table 4. 4: Seroprevalence and animal level factors associated with *C. burnetii* Infection – univariate analysis

Variable	n (%)	Positive samples (%)	OR(95% CI)	p - value
Species				
Goat	508(60.5)	132(25.9)	2.6 (1.7 - 3.7)	<0.001
Sheep	332(39.5)	40(12.1)	1†	
Breed				
Indigenous	723(86.1)	158(21.9)	2.1 (1.1 - 3.6)	0.019
Cross breed	117(13.9)	14(12.0)	1†	
Sex				
Female	680(80.9)	150(22.1)	1.7 (1.1 - 2.8)	0.02
Male	160(19.1)	22(13.8)	1†	
Age group				
≤1 year	168(20)	18(10.7)	1†	-
> 1 - 2 years	236(28)	49(20.8)	2.2 (1.2 - 3.9)	0.01
> 2 - 3 years	197(23.5)	39(19.8)	2.1 (1.1 - 3.7)	0.03
> 3 - 4 years	119(14.2)	24(20.2)	2.1 (1.1 - 4.1)	0.04
> 4 years	120(14.3)	42(35.0)	4.5 (2.4 - 8.3)	<0.001
Production system				
Mixed farming	303(41.8)	42(13.9)	1†	-
Agro-pastoralism	351(36.1)	59(16.8)	1.3 (0.8 - 1.9)	0.35
Nomadic pastoralism	186(22.1)	71(38.2)	3.8 (2.4 - 5.9)	<0.001
Sub location	102 (12.2)	11(10.8)	1†	
Eldume	133 (15.8)	15(11.3)	1.1(0.4, 2.6)	0.912
Endao	60(7.1)	11(18.3)	1.9(0.7, 5.0)	0.221
Maji Ndege	125(14.9)	17(13.6)	1.3(0.5, 3.4)	0.586
Ngambo	72(8.6)	16(22.2)	2.4(0.9, 6.1)	0.078
Perkerra	186(22.1)	71(38.2)	5.1(2.3, 11.4)	<0.001
Salabani	162(19.3)	31(19.1)	2.0(0.8, 4.5)	0.115
Sandai				

†- Reference level, CI – Confidence Interval, OR- Odds Ratio

On multivariable analysis after fitting the mixed effect models with household herd as a random effect, the relationship between *C. burnetii* seropositivity and putative risk factors was evaluated. The Intra-cluster correlation coefficient (ρ) determined was 0.25. Nomadic pastoralism was associated with higher odds for sero-positivity OR 2.6 (95% CI: 1.6, 4.0); p<0.001 compared to non-nomadic pastoralism systems.

In addition, goats had a higher likelihood of being seropositive OR 2.2 (95% CI: 1.4, 3.4); $p < 0.001$ when compared to sheep, and likelihood of seropositivity increased with age (Table 4.5).

Table 4. 5: Animal Level Factors Associated with *C. burnetii* Infection, Mixed – effect Multivariable Logistic Regression

Variable	OR	95% CI	*p - value*
Production system			
Non-nomadic pastoralism	1.0†		
Nomadic pastoralism	2.6	1.6, 4.0	<0.001
Species			
Sheep	1.0†		
Goat	2.2	1.4, 3.4	<0.001
Age group			
≤1 year	1.0†		
> 1 - ≤2 years	2.1	1.2, 4.0	0.015
> 2 - ≤3 years	2.0	1.1, 3.9	0.032
> 3 - ≤4 years	2.3	1.1, 4.7	0.021
> 4 years	4.0	2.1, 7.8	<0.001
Sub location			
Eldume	1.0†		
Endao	1.0	0.4, 2.5	0.969
Maji Ndege	1.5	0.5, 4.3	0.462
Ngambo	1.6	0.5, 4.8	0.434
Perkerra	2.4	0.9, 6.6	0.084
Salabani	-	-	-
Sandai	2.6	1.1, 6.3	0.028

†- Reference level, OR – Odds Ratio, CI – Confidence Interval

4.5 Herd Seroprevalence and Associated Herd Level Factors

Of the 140 households enrolled, 92(66% 95% CI: 57.6, 73.2) had at least one animal seropositive. Households practicing nomadic pastoralism were more likely to be *C. burnetii* seropositive compared to those practicing agro-pastoralism and mixed farming production systems (OR= 7.6, 95% CI: 2.3-34.1), p = 0.0023. Recent introduction of a new animal in the herd, herd contact with other herds or wild animals, tick control and the frequency of tick control were not significantly associated with *C. burnetii* herd seropositivity (Table 4.6). Additionally there were no differences in *C. burnetii* herd seropositivity in households that reported reproductive disorders and those which did not (Table 4.6).

Table 4. 6: Herd seroprevalence and herd level factors associated with *C. burnetii* herd positivity

Variable	% seroprevalence (95% CI)	Odds ratio (95% CI)	p - value
Introduced a new animal			
Yes	61.9(40.2 - 80.5)	0.79(0.3 - 2.1)	0.6351
No	67.2(58.4 - 75.2)		
Contact with other herds			
Yes	66.6(58.5 - 74.2)	0.5 (0.03 - 8.17)	0.6214
No	50(25 - 97.5)		
Contact with wild animals			
Yes	69.6(61.5 - 77.0)	0.5 (0.03 - 8.17)	0.6214
No	12.5(0.6 - 48.0)		
Tick control			
Yes	66.2(57.9 - 73.7)	0.6 (0.02 - 6.3)	0.7136
No	75(24.2 - 98.8)		
Tick control frequency			
Weekly	47(24.2 - 70.3)	1.1(0.7 - 1.6)	0.47
After 2 weeks	68.5(57 - 78.6)		
Monthly	71.8(54.6 - 85.3)		
Quarterly	64.7(40.5 - 84.3)		
Bucks bred other herd does			
Yes	68.8(59.6 - 77.1)	1.5(0.5 - 3.9)	0.4393
No	60(37.8 - 79.4)		
Rams bred other flock ewes			
Yes	65.5(55.1 - 74.9)	0.8(0.4 - 1.8)	0.7647
No	68.1(53.8 - 80.2)		
Reproductive disorders			
Abortions	64.8(54.8 -74.0)	0.8(0.4 - 1.7)	0.5838
Still births	83.3(40.9 - 99)	2.6 (0.3 - 23.0)	0.3718
Weak young’s	65.5(53 - 76.6)	0.9 (0.4 - 1.9)	0.8512
Infertility	75(24.3 - 98.8)	1.5 (0.2 - 15.1)	0.7136
Retained placenta	62.5(37.6 - 83.2)	0.8 (0.3 - 2.4)	0.7246
Herd production system			
Mixed farming	54.9(41.2 - 68.1)	1	-
Agro-pastoralism	63.8(50.8 -75.4)	1.4 (0.7 - 3.1)	0.3459
Nomadic pastoralism	90.3(75.8 - 97.5)	7.6 (2.3 - 34.1)	0.0023

OR – Odds Ratio, CI – Confidence Interval

4.7 Practices that may Expose Humans to Zoonoses in Baringo County

Of all the respondents interviewed, majority 86% (n=120) reported to have assisted animals during giving birth in the last six months. About 82% (n=115) and 78% (n=109) respondents reported to have participated in assisting in removal of retained placenta and had contact with aborted fetuses in the last six months respectively. Use of Personal Protective Equipment (PPEs) was very minimal with only 3% (n= 4) reporting to have used PPEs during contact with animals (Table 4.7).

Table 4. 7: Practices that may expose humans to zoonoses in Baringo County

Variable	No. of Respondents n (%)
Assisted animals during births in the last six months	
Yes	120(85.7)
No	20(14.3)
Assisted in removal of retained placenta in the last six months	
Yes	115(82.1)
No	25(17.9)
Contact with aborted fetuses in the last six months	
Yes	109(77.9)
No	31(22.1)
Use PPEs during contact with animals	
Yes	4(2.9)
No	136(97.1)

4.8 Knowledge on Zoonoses among the Study Participants

Majority of the respondents, 75% (n=106) had information on existence of diseases transmitted between animals and humans (zoonoses). About 75% (n=105) knew rift valley fever disease while only 5% (n=7) had information on Q fever. The most common source of information on zoonoses was public health officers at 64% (n=90), followed by veterinary officers 58% (n=81) while the least was from community health workers and local administrators 2% (n=3).

In terms of information on how zoonoses are transmitted, 65% (n=91) believed drinking raw milk was the main mode of zoonosis transmission while only 6% (n=8) believed living in close proximity to animals could lead to exposure to zoonotic diseases. Half of the respondents 50% (n=70) indicated that milk pasteurization could prevent acquisition of zoonoses in humans while only 14% (n=19) believed use of PPEs when in contact with animals can actually prevent zoonoses in humans (Table 4.8).

Table 4. 8: Knowledge on Zoonoses among the Study Participants

Variable	No. of Respondents n (%)
Do you know diseases people can get from animals (Zoonoses)	
Yes	106(75.4)
No	34(24.6)
Zoonoses respondents have heard of	
Rift valley fever	105(75)
Brucellosis	94(67.1)
Anthrax	54(38.6)
Rabies	46(32.8)
Q fever	7(5)
Source of information on zoonoses	
Public Health Officers	90(64.3)
Veterinary officers	81(57.8)
Radio	14(10)
Public barazas	7(5)
Newspaper	4(2.8)
Community health workers	3(2.1)
Local administrators	3(2.1)
Modes of zoonoses transmission to humans	
Assisting animals during delivery without PPEs	43(30.7)
Handling aborted fetuses without PPEs	28(20)
Drinking raw milk	91(65)
Living in close proximity with animals	8(5.7)
Methods of zoonoses prevention	
Milk pasteurization	70(50)
Use of PPEs when in contact with animals	19(13.6)
Vaccination of animals	64(45.7)
Public health education	54(38.6)

Knowledge on disease assessed by participant's description of disease symptoms. Multiple responses were allowed hence the proportion is calculated for each response.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 *Coxiella burnetii* seroprevalence

Seroprevalence of *C. burnetii* antibodies in small ruminants both at individual animal and herd level was reported in the study area. At animal level, overall 20.5% of the small ruminants sampled were found to have *C. burnetii* antibodies. At herd level, the probability that a randomly selected herd has at least one animal exposed to *C. burnetii* was high with seven out of every ten herds selected having at least one animal seropositive.

In this study in rural Baringo County region, Kenya 25.9% of goats and 12.1% of sheep had antibodies to *C. burnetii*. Although it is slightly low than the 32% in goats and 18.2% in sheep detected in a recent study in rural western Kenya, the studies show a similar trend where goats are significantly associated with *C. burnetii* seropositivity compared to sheep (Knobel *et al*., 2013).

A critical literature review on the prevalence of *C. burnetii* infection in domestic ruminants in several countries globally showed a wide variation in reported prevalence and the quality of the studies contacted (Guatteo, Seegers, Taurel, Joly, & Beaudeau, 2011). In that review, the estimated average prevalence on animal level were 15% and 27% for sheep and goats respectively (Guatteo *et al*., 2011). However, of the 69 published papers reviewed, only four studies on domestic small ruminants were carried out in Africa, which highlights the significant gap in *C. burnetii* burden and risk analysis studies in Africa despite the disease being highly endemic globally (Guatteo *et al*., 2011).

A study in Chad, Central Africa, a region with almost similar production system as the study area in this study, seroprevalence of 11% and13% were found in sheep and goats respectively (Schelling *et al*., 2003). Results of this study showed a higher

seroprevalence among small ruminants, which implies that Q fever is of considerable importance in the small ruminant population of Kenya.

In this study an indirect IgG ELISA was used to detect the presence of antibodies against *C. burnetii*. The choice of serology as the test method has a limitation in that, presence of antibodies may not be evidence of active *C. burnetii* infection but only shows that an animal has been previously exposed to *C. burnetii* (Muskens, van Engelen, van Maanen, Bartels, & Lam, 2011; Sidi-Boumedine *et al*., 2010) .

This limits the interpretation of the results at individual animal level as presence of these antibodies indicates past exposure and may not be evidence of current infection or actual shedding of bacteria as animals may seroconvert without detectable shedding and may also remain seropositive for a long time after infection has resolved while other animals shed bacteria but do not seroconvert (McQuiston, Childs, & Thompson, 2002). However, serology is a suitable technique for screening herds as it provides insights on the level of exposure to the pathogen in the region cost effectively (EFSA Panel on Animal Health and Welfare (AHAW), 2010).

5.1.2 Animal Level Factors Associated with *Coxiella burnetii* Infection

Associations between past *C. burnetii* infection and age, species (goats versus sheep), and production system were seen. Age was found to be significantly associated with *C. burnetii* seropositivity with older animals being more likely to be seropositive compared to young animals. The difference was more pronounced when animals less than one year were compared to animals aged more than four years, where animals more than four years were four times more likely to be exposed compared to animals less than one year. The results are consistent with previous studies which have shown seropositivity to increase with age, with peak prevalence being achieved at reproductively mature animals at 2 – 3 years (Knobel *et al*., 2013; Van den Brom, Moll, van Schaik, & Vellema, 2013). This is attributed to the horizontal nature of *C. burnetii* transmission and the exposure to the pathogen continuously from young age due to the livestock production system in this setting where animals are allowed to roam freely during grazing.

Goats had two-fold higher likelihood of exposure to infection compared to sheep. Varying seroprevalence in sheep and goats has been documented with some studies documenting higher seroprevalence in goats (Klaasen *et al.*, 2014; Knobel *et al.*, 2013), while others recording higher seroprevalence in sheep (Ruiz-Fons *et al.*, 2010). The difference in inherent susceptibility to *C. burnetii* between sheep and goats has not been previously demonstrated and hence this needs further research.

Nomadic pastoralism was associated with almost a threefold increase in the likelihood of being *C. burnetii* seropositive compared to non-nomadism. This could be attributed to the extensive mobility and contact between different pastoral herds during grazing and aggregation in common watering points, compounded by the fact that *C. burnetii* persists and remains infectious in the environment for months to years (Raoult *et al.*, 2005).

The study utilized a cross-sectional study design which may not be entirely suitable for investigation of putative risk factors of the pathogen as it does not allow the investigator to determine the time of first infection or introduction of the pathogen to the herd and hence may pose a danger of misclassification of the risk factors associated with seropositivity. However, the aim of the study was to identify associations and not cause effect relationships hence the design was considered sufficient to identify the factors with significant associations to the study outcome.

5.1.3 Herd Level Factors Associated with *Coxiella burnetii* Infection

Of all the herd level factors analyzed, livestock production system was the most significant risk factor for herd seropositivity. The difference was quite distinct between herds under non-nomadic pastoralism system and those under nomadic pastoralism management system. Herds under nomadic pastoralism production system were 7.6 times more likely to be seropositive compared to those under non-nomadic pastoralism system. This could be attributed to the extensive system of production in nomadic pastoralism which is associated with free roaming movements, common grazing and watering points which may have contributed to

disease transmission and spread among the herds through increased risk of livestock contact.

Studies elsewhere have documented the association between *C. burnetii* and reproductive disorders in small ruminants (Vaidya *et al*., 2010). However, in this study no association was found. The random selection of animals to be sampled from the herds may explain the lack of association found between *C. burnetii* herd seropositivity and history of occurrence of reproductive disorders in the herd. The lack of association was also observed in another study in northern Spain (Ruiz-Fons *et al*., 2010).

5.1.4 Practices that may Expose Humans to Zoonoses in Baringo County

Majority of the respondents reported to have had direct contact interaction with their animals and performed tasks such as assisting in animal births and removal of retained placenta without use of personal protective equipment. Although a simultaneous assessment of human and animal serology for *C. burnetii* in this study was not conducted, zoonotic implications in this setting are plausible.

5.1.5 Knowledge on Zoonoses among Study Participants

Knowledge on zoonoses was varied with seventy five percent of the respondents being aware of existence of zoonoses. However there is need for sensitization to the twenty five percent of the population who had no information on the threat of zoonoses exposure. Of the zoonoses, the respondents were familiar to, majority knew of rift valley fever disease while very few (5%) had information on Q fever. This could be attributed to the fact that some high profile rift valley fever outbreaks have occurred in Kenya with resultant heavy economic burden (Murithi *et al*., 2011; Rich & Wanyoike, 2010). The findings indicate both public health officers and veterinary officers were an important source of advice and knowledge on zoonoses. Most of the respondents believed that milk pasteurization, vaccination of animals, and public health education are critical strategies for prevention of zoonoses.

5.2 CONCLUSIONS

1. The overall seroprevalence of *Coxiella burnetii* infection at animal level was 20.5% while at herd level it was 66 %.
2. The animal-level factors associated with *C. burnetii* infection were age, species (goats versus sheep), and production system.
3. The farm-level factors (livestock management practices) associated with *C. burnetii* infection was nomadic pastoralism system.
4. Majority of the respondents reported to have had direct contact interaction with their animals and performed tasks such as assisting animals during parturition and removal of retained placenta without use of personal protective equipment. These practices may expose the residents to zoonoses in cases of infected animals.

5.3 RECOMMENDATIONS

1. The Zoonotic Disease Unit in collaboration with the County government of Baringo should conduct further study to identify the presence of active infection in domestic ruminants and humans using direct diagnostic techniques in order to evaluate the true risk posed by domestic ruminants as a source of *C. burnetii.*
2. To reduce the impact of the disease in these populations, Baringo County government should implement public health measures aimed at mitigating the risk of transmission in both domestic ruminants and humans.
3. To increase the level of knowledge and awareness of Q fever and other zoonoses in this setting, awareness campaigns should be carried out to sensitize the community on Q fever and other zoonoses etiology, transmission dynamics, prevention and control strategies.

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APPENDICES

Appendix 1: Animal Factor Data Collection and Sample Tracking Tool

Animal Factor Data Collection and Sample Tracking Tool/Questionnaire

Sub-county: **Location:** **Sub-location:**

Household ID: **Date:** **Production system:**

#	Specimen ID	Species	Age group	Sex	Breed	Results
1.						
2.						
3.						
4.						
5.						
6.						
KEY		1=Goat 2=Sheep	1 = ≤1 year 2 = 1 – 2 years 3= 2 – 3 years 4 = 3 – 4 years 5 = >4 years	M = Male F = Female	1 = Indigenous/local 2 = Improved/Mixed	1= Pos 2=Neg

Appendix 2: Informed Consent Form

Title of study:

Sero-prevalence and associated factors for *Coxiella burnetii* infection in sheep and goats in Baringo County

Introduction:

My name is Josphat Mulei Muema. We are visiting your household to learn more on diseases of livestock which can be spread from livestock to humans such as Q fever. People get most of these diseases when exposed to infected livestock and livestock products.

Purpose of study:

The aim of this study is to find out how many of your animals and others in this area and other areas in Baringo County are exposed to this disease, and what are the things which contribute to animals getting the disease. The information gathered will help in designing ways of disease control and prevention in the area and the country. You are being requested to participate in this study because your herd was picked by chance among other herds in this area.

Expectations of the study:

If you agree to participate in the study, we will remove blood samples (one table spoon) from some of your animals and test in laboratory to check if they are infected with Q fever. I shall then ask you some questions which are written on a paper on how you take care of your animals. The test results shall be availed as soon as possible to County Veterinary Officer who shall forward them to you and advice on any necessary control measures if need be.

Risks:

We don’t foresee any risks from participating in this study. However minor bruising and bleeding may occur on the selected sheep and goats during sample collection.

Benefits:

The study results will be shared with the concerned authorities for them to take action on the study recommendations including necessary control measures if need be.

Confidentiality:

Any information obtained from you will be kept confidential and used only for the purposes of this study. The results of this research may be published in scientific journals or presented at medical or veterinary meetings, but your identity will be concealed.

Compensation:

If you accept to be part of this study, there will be no payment for participation.

Alternatives:

You have a free choice to agree or to decline to participate in this study. If you agree to participate in the study you are also free to withdraw from the study at any time if you so wish without any consequences whatsoever.

Approval of the study:

This study has been approved by:

The ILRI Institutional Research Ethics Committee (IREC)

Email: irecbox@cgiar.org

And

Board of Post graduate studies

Jomo Kenyatta University of Agriculture and Technology

P.O. Box 62,000, Juja, Kenya

In case of any further questions or concerns, you can address them to the directors of the above institutions.

Consent:

This study has been fully explained to me, the risks and benefits of it. I had the opportunity to ask questions which were satisfactorily answered. I therefore consent to voluntarily participate in the study.

Name of participant ……………………………………………………………..

Signature/thumb print of participant………………………………………………..

Date……………………………………………………………..…………..

Name of researcher/research assistant …………………………………………

Signature………………………………….. Date …………………………….

Appendix 3: Household Questionnaire

PART 1: HOUSEHOLD INFORMATION

To be answered by the household head

Serial no………………………………….

A. General Information			
Date (dd/mm/yy):		**Enumerator's Name:**	
County:		**Sub –county:**	
Division:		**Location:**	
Sub-location:		**Village:**	

GPS Coordinate of the household...

Latitude	Longitude	Elevation

B. Household demographics			
Household ID (HHID):		**Number of household members:**	
House hold Head's Name (*at least two names*):			
Telephone:			
Enter information on the table below on household head			
Age	**Sex**	**Highest level of completed Formal education**	**Occupation**

(Years)	1=Male 2=Female	0=No formal education 1 = Primary 2 = Secondary 3 = Tertiary 4= Adult education	1=Employed full time on farm 2 =Employed part time on farm 3 =Self-employed off farm 4= Employed off farm - agriculture 5= Salaried off farm 6 =Other (Specify)

C. Animal demographics

C1. Do you own any livestock (Sheep, and Goats)? ○ Yes ○ No

a) If yes, is the herd owned by ○ Man ○ Woman ○ Jointly owned

C2. How many animals of each species do you own?

Livestock	**C3. Number Owned**	**C4. Breeds**	**C5 Production system**	**C6. Main source of water**	**C7. Breeding system**
Sheep					
Goats					

Breeds:	1=Indigenous;	2.= Exotic;	3 = Cross
Production system:	1=Nomadic pastoralist; 3=Mixed farming		2=Agro-pastoralist;
Water source:	1=Pan/lake	2=Borehole, 3= River	4 = Tap water
	5= Other (Specify)		
Breeding system:	1 = Natural	2 = Don't breed	

C 8.	Who else owns animals in this herd?	○Neighbor	○ Relatives
	○ Friends	○Other (Specify)	

D. PART III: HERD INFORMATION

To be answered by the household head or the person taking care of the animals

	Risk factor information		
D1.	Has your herd come in contact with other herds during grazing or watering in the past six months?		
	○ Yes	○ No	○ Don't know
D2.	Has your herd come in contact with wild animals during grazing or watering in the past six months?		
	○ Yes	○ No	
	D2.1 If yes which wild animals?(list all)		

D3.	Have you experienced any of the following in your livestock in the past six months? (check all that apply)		
	○ Abortions	○ Still births	○ Weak young
	○ Infertility	○ Retained placenta	

D4.Have your bucks bred does belonging to another herd in the last six months?	○ Yes	○ No
D5. Have your rams bred ewes belonging to another herd in the last six months?	○ Yes	○ No
D6. Do you use designated areas when your does give birth?	○ Yes	○ No
D7. Do you use designated areas when your ewes give birth?	○ Yes	○ No
D8. Have you ever found aborted fetuses on the grazing pastures in the last six months?	○ Yes	○ No
D9. Have you ever found aborted fetuses around the watering point in the last six months? D10 Do you do tick control in your herd? ○ Yes ○ No D11. If yes, which methods do you use? ○ Dipping ○ Spraying ○ Handpicking ○ Hand dressing, ○Others specify……………………… D12. If you do dipping/spraying, how far do you move your	○ Yes	○ No

animals for dipping/spraying? (km) ○ less than a km ○ Between 1 -5 km ○ More than 5 km D13. How often do you do the tick control method? ○ Weekly ○ After Two weeks ○ Monthly ○ Quarterly ○ Yearly D14. Which tick control products do you use? ○ Commercial acaricides ○ Traditional herbs ○Others specify…………………………………………… D15. What is the total cost of tick control in your herd per month ? ……………………………….. D16. What is the source of milk consumed in this house hold? ○ Own animals ○ Neighbors’ animals ○ Purchase pasteurized milk D17. How is the milk consumed in your household? ○ Raw ○ Boiled ○ Both		

D18. Have you or any member of your household assisted animals during births in the last six months? ◯ Yes ◯No D19. Have you or any member of your household assisted in removal of retained placentas in the last six months? ◯ Yes ◯ No D20. Have you or any member of your household had contact with aborted fetus in the last six months? ◯Yes ◯No		

D21. During interaction with animals, do you and other household members use protective gear? ◯Yes ◯ NO

D22. Have you introduced any new animal in your herd, either through purchase or any other means in the last six months? ◯Yes ◯No

E. PART IV: SOCIO - ECONOMICS

To be answered by the household head or the person taking care of the animals

---

E1. What is the estimated total monthly income in your household (kshs)?

◯ Less than Kshs 10,000

◯ Between Kshs 10,000 – 20,000

◯ Above Kshs 20,000

E2. What is the type of residence/ housing in your household?

○ Temporaly (walls are mud)

○ Semi-permanent (walls are iron sheets or timber)

○ Permanent (walls are stone or bricks)

E3. What is the source of fuel used in this household?

○ Fire wood

○ Paraffin

○ Gas

○ Electricity

E4. What is the estimated monthly cost of disease control in your herd? (Kshs)………..

F. PART V: FARMER'S KNOWLEDGE

To be answered by the household head or the person taking care of the animals

---

F1. Have you heard of diseases people can get from animals (sheep and goats) ○Yes ○ No

F2. If yes which ones? (check all that are mentioned)

○ Q fever

○ Brucellosis

○ Rift valley fever

○ Anthrax

○ Rabies

◯ Others specify____

F3. If yes, where did you get the information from?

◯ Public health officers

◯ Veterinary officers

◯ Radio

◯ Megaphone

◯ Barazas

◯ Newspapers

◯ Community health workers

◯ Local administrator

F4. In your opinion, which animals are affected by the disease(s) you mentioned above (F2)? (Check all that are mentioned.)

◯ Goats

◯ Sheep

◯ Cattle

◯ Camels

◯ Dogs

◯ Cats

◯ Poultry

◯ Donkeys

Others (specify)……………………………………………………

F4. In your opinion, what are the common signs and symptoms of the disease(s) you mentioned above (F2) in animals?

◯ Abortions

◯ Still births

◯ Birth of weak young ones

◯ Infertility

◯ Others specify____

F5. Do you know whether animals can transmit the disease(s) mentioned in F2 to humans?

◯ Yes ◯ No ◯ Don't know

F6.If yes above, how is it transmitted from animals to humans? (Check all that are mentioned.)

◯ Helping animals to deliver/abort by bear hands

◯ Handling fetal tissues/aborted fetuses with bear hands

◯ Drinking raw milk

◯ Consuming products processed from raw milk

◯ Milking animals

◯ Living with animals

Others (specify) ……………………………………………………………

F7. Do you know the signs and symptoms of the disease(s) mentioned in F2 in humans?

◯ Fever (hotness of body)

◯ Headache

◯ Body weakness

◯Aching of muscles/joints

◯Dry cough

◯Sore throat

◯Chest pain

◯Abdominal pain

Others……………………………………………………………………………………
……………………………

F8. Do you know how the disease(s) mentioned in F2 it can be prevented? (check that apply)

◯ I don't know

◯ Boiling/pasteurized milk

◯ Not handling birth products without Personal Protective Equipment.

◯ Removing/treating ticks on animals

◯ Public health education

◯ Vaccination of animals

◯ Others specify____

Appendix 4: ERC Ethical Approval

19 January 2015

Our Ref: ILRI-IREC2014-14

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

Dear Josphat Mulei,

RE: SERO-PREVALENCE AND ASSOCIATED FACTORS FOR *COXIELLA BURNETII* INFECTION IN SHEEP AND GOATS IN BARINGO COUNTY

Thank you for submitting your research proposal to the ILRI Institutional Research Ethics Committee (ILRI IREC).

This is to inform you that ILRI IREC has reviewed and approved the study titled ***'Sero-prevalence and associated factors for Coxiella burnetii infection in sheep and goats in Baringo County***. The approval period is January 19, 2015 to January 31, 2016. The approval 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.

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

Yours Sincerely,

Delia Grace

Delia Grace
Chair, ILRI Institutional Research Ethics Committee

Documents received & reviewed:
- Research Project Compliance Form
- IREC Form 1 (Brief)
- Project Proposal
- Interview Guide
- Household Questiinaire
- Consent Form
- Animal Factor Data Collection and Sample Tracking Tool

Appendix 5: Published Manuscript

Zoonoses and Public Health

ORIGINAL ARTICLE

Seroprevalence and Factors Associated with *Coxiella burnetii* Infection in Small Ruminants in Baringo County, Kenya

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[3] College of Health Sciences, Jomo Kenyatta University of Agriculture & Technology, Nairobi, Kenya
[4] Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, USA
[5] Center for Global Health Research, Kenya Medical Research Institute, Kenya
[6] Directorate of Veterinary Services, Nairobi, Kenya
[7] Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Kenya
[8] Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, USA
[9] Zoonotic Disease Unit, Department of Preventive and Promotive Health Services, Ministry of Health, Nairobi, Kenya

Impacts

- The study aimed to determine the seroprevalence and factors associated with *Coxiella burnetii* infection in small ruminants' population in Baringo County, Kenya, following reports of acute cases of Q fever in humans.
- Our results demonstrated a high seroprevalence of *C. burnetii* infection in the small ruminants' population. Nomadic pastoralism, goats and being older than 1 year of age were associated with greater risk of *C. burnetii* seropositivity.
- Our study provides insights on *C. burnetii* circulation in the study population, correlated factors and provides a basis for instituting prevention and control strategies.

Keywords:
Seroprevalence; *Coxiella burnetii*; Q fever; small ruminants; baringo; Kenya

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Summary

To improve estimates of *C. burnetii* epidemiology in Kenya, a survey was undertaken in small ruminants in Baringo County, where acute cases of Q fever in humans had been reported in 2014. From 140 household herds selected, 508 (60.5%) goats and 332 (39.5%) sheep were included and an indirect ELISA assay for *C. burnetii* IgG antibodies performed. In addition, epidemiological information at both herd and animal level was collected. Generalized mixed-effects multivariable logistic model using herd as the random effect was used to determine variables correlated to the outcome. Overall seroprevalence was 20.5% (95% CI: 17.8%, 23.3%). Goats had 26.0% (95% CI: 22.2%, 30.0%) compared to sheep 12.2% (95% CI: 8.7%, 16.0%). Nomadic pastoralism, goats and older animals (>1 year) were associated with greater risk of *C. burnetii* seropositivity ($P = \leq 0.05$). Heterogeneity in *C. burnetii* seropositivity was observed across the sublocations ($P = 0.028$). Evidence of *C. burnetii* exposure in small ruminants revealed poses a potential risk of exposure to the people living in close proximity to the animals. We recommended integrated animal–human surveillance and socio-economic studies for *C. burnetii*, to aid our understanding of the risk of transmission between the animals and humans, and in the design of prevention and control strategies for the disease in the region.

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Introduction

Q fever is a highly endemic zoonosis with worldwide distribution except in New Zealand (Hilbink et al., 1993). The disease is caused by the obligate intracellular Gram-negative bacterium, *Coxiella burnetii* (Gurtler et al., 2014). Domestic small ruminants are the main reservoirs of *C. burnetii*, although its host range is varied including wild and domestic ruminants, dogs, cats, birds, arthropods and reptiles (Maurin and Raoult, 1999).

Infection is primarily through inhalation of aerosolized contaminated materials from infected livestock, especially parturient animals and their products or their contaminated environment which makes the disease an occupational zoonosis (Battelli et al., 2006; Porter et al., 2011; Schimmer et al., 2012; Van den Brom et al., 2013b). However, Q fever outbreaks in urban areas in people with no history of contact with livestock or livestock products have occurred (Amitai et al., 2010), and exposure attributed to *C. burnetii* resistant and persistent nature to harsh environmental conditions for months to years (Gurtler et al., 2014).

Airborne transmission of *C. burnetii* has been reported where the bacterium is carried by wind resulting in Q fever outbreaks (Hawker et al., 1998; Tissot-Dupont et al., 1999, 2004). Livestock trade and animal movement contribute to *C. burnetii* transmission and spread dynamics between herds (Nusinovici et al., 2015; Pandit et al., 2016). Local environmental conditions such as vegetation and soil moisture play an important role in *C. burnetii* transmission from infected farms (van der Hoek et al., 2011). Transmission by indirect contact through aerosolization of the bacterium has also been documented as reported in the 2007–2010 Netherlands outbreak (van der Hoek et al., 2010; Schimmer et al., 2010, 2011; Roest et al., 2011).

In humans, the disease presents in an acute or chronic form; with the acute disease being characterized by fever, atypical pneumonia and hepatitis while the chronic form manifests with long-term sequelae including fatigue, abortions and heart disease (Anderson et al., 2013; Wielders et al., 2014). Q fever associated mortality is usually low (1–2%) in treated patients (Tissot Dupont et al., 1992), but the mortality may be as high as 65% in infected persons who develop chronic disease (Raoult et al., 2005).

In domestic ruminants, the disease is characterized by reproductive disorders including abortions, stillbirths, premature delivery and birth of weak offsprings (Vaidya et al., 2010; Cantas et al., 2011; Agerholm, 2013). Domestic small ruminants pose public health risk of Q fever outbreaks as was documented in the 2005 outbreak in Germany, where 331 human cases were associated with sheep grazing and lambing near a residential area (Gilsdorf et al., 2008) and in the Netherlands outbreak between 2007 and 2010 where over 4000 human cases were associated with exposure to goat herds (Delsing et al., 2010).

In spite of the notable nature of some of the Q fever outbreaks, and the global health security risk that *C. burnetii* poses as a potential bioterrorism agent (Madariaga et al., 2003), data on the epidemiology of *C. burnetii* infection in sub-Saharan Africa are largely unavailable as most of Q fever risk analysis studies have been conducted in the developed countries in Europe were the livestock rearing system is different from the ones practiced in resource poor settings in sub-Saharan Africa (Dupont et al., 1995; Georgiev et al., 2013; Vanderburg et al., 2014).

A systematic review of *C. burnetii* epidemiology across Africa revealed its endemicity in cattle, small ruminants and humans across the continent, with seroprevalence ranging from 7% to 33% in sheep, 4% to 55% in cattle and 1% to 32% in humans (Vanderburg et al., 2014).

In Kenya, evidence of *C. burnetii* exposure has been reported since 1950s, where the prevalence of *C. burnetii* antibodies in humans was reported to range between 10% and 20% (Njeru et al., 2016). In an investigation of a group of 50 travellers for a safari trip to Kenya, four people (8%) were found to have contracted Q fever (Potasman et al., 2000). Among domestic ruminants in Kenya, previous studies have documented seroprevalence ranging from 7% to 57% in cattle and 33% to 34% in goats (Njeru et al., 2016). In rural western Kenya, *C. burnetii* antibodies were detected in 30.9% of humans, 28.3% of cattle, 32.0% of goats and 18.2% of sheep (Knobel et al., 2013). A study in Laikipia County showed a distinct seroprevalence gradient, with seroprevalence ranging from 0% to 4% in cattle, 13% to 20% in sheep, 31% to 40% in goats and 5% to 46% in camels (DePuy et al., 2014). A recent linked human–cattle population survey in western Kenya detected prevalence of 2.5% in humans and 10.5% in cattle (Wardrop et al., 2016).

In 2014, cases of acute Q fever in humans were reported in Baringo County, Kenya, with 54.8% of human samples collected during an outbreak investigation being positive for anti-phase II antibodies and confirmed through quantitative polymerase chain reaction (qPCR) [Baringo County Health department, unpublished data]. In Baringo County, the main livestock rearing systems practiced are mainly nomadic pastoralism and agro-pastoralism. These systems are characterized by free roaming, common grazing, watering points and live animal markets which pose various challenges in enhancing biosecurity measures. These factors contribute to increased frequency of livestock contact which in turn increase the risk of disease transmission and spread between herds. This system being different from the more sedentary livestock production system in Europe where most *C. burnetii* risk analysis studies have been

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conducted highlights the importance of *C. burnetii* epidemiological studies in these less studied systems, which might provide new and important insights on *C. burnetii* epidemiology including intra- and inter-herd transmission dynamics. Our study aimed at determining the seroprevalence of *C. burnetii* in goats and sheep from the same region in Baringo where acute cases of Q fever had been reported in humans and investigating the factors associated with seropositivity in Baringo County.

Materials and Methods

Study area and population

This study was conducted between 13 April and 27 May 2015 in Baringo County, Kenya (Fig. 1). Baringo is partially an arid and semi-arid county situated in Rift Valley. It borders Turkana and Samburu to the north, Nakuru to the south, Laikipia to east, West Pokot, Elgeyo Marakwet, Nandi, Kericho and Uasin Gishu to the west. The rainfall pattern in the area is bimodal with long rains falling between March and May, and short rains between August and November. The county covers an area of 11 015 square kilometres, which includes 195 km^2 of lakes Baringo and Bogoria. It has an estimated population of 555 561 and 110 649 households (Kenya National Bureau of Statistics, 2010).

The main economic activity is livestock production especially small ruminants whose population is estimated to be 482 706 sheep and 1 771 833 goats (Kenya National Bureau of Statistics, 2010). The main livestock rearing systems practiced are mainly nomadic pastoralism and agro-pastoralism involving free roaming animals in search for pasture and water. Livestock marketing is mainly through live animal markets/auction where the county is associated with the largest small ruminant live market/auction, 'Kimalel goat auction' (Beneah et al., 2014). Administratively the

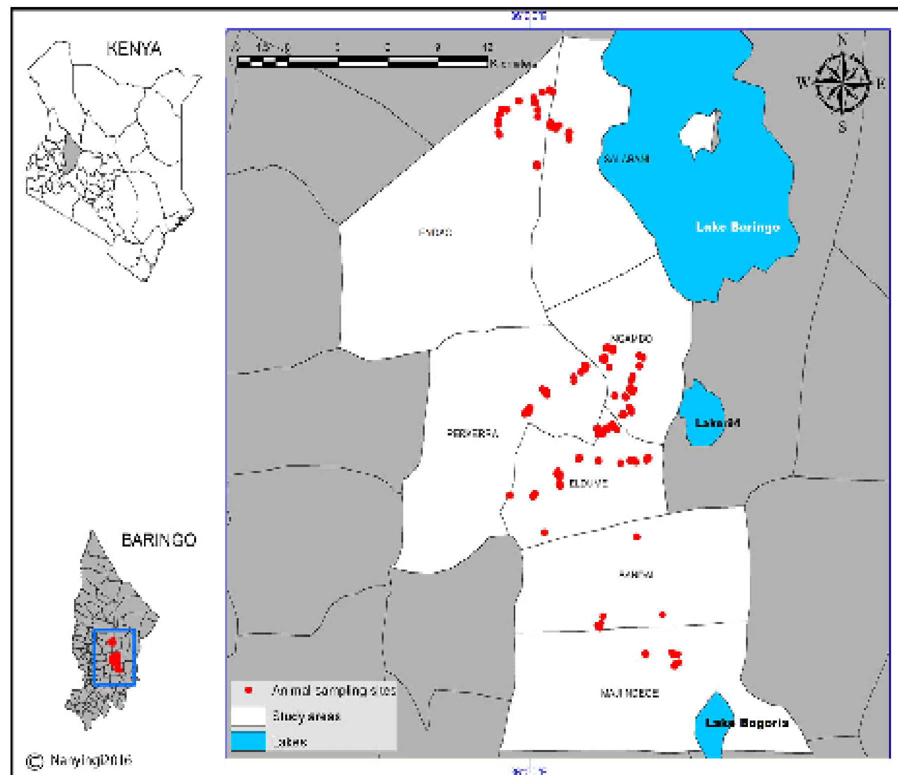

Fig. 1. Geographical location of study sites in Baringo County, Kenya, indicating all sampled households (red dots). [Colour figure can be viewed at wileyonlinelibrary.com].

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county is divided into six subcounties namely Mogotio, Koibatek, Baringo South (Marigat), Baringo Central, Baringo North and East Pokot (Tiaty).

Study design, sampling unit, sampling strategy and sample size calculation

The study design was cross-sectional based on multilevel random sampling design. The sampling frame was based on sheep and goat herds within the seven administrative sublocations that formed the study area. Household herds were the primary sampling units while the individual animals within the herds were the secondary sampling units. A household herd was defined as ownership of one or more animals of either of the study species. Household heads or their representatives participated by providing epidemiological information both at animal and herd level.

Our study focused mainly on two subcounties, Baringo South (Marigat) and Baringo North that represent varied production systems including, mixed farming, pastoral, agro-pastoral and irrigated agriculture. In the two subcounties, seven sublocations (Endao, Salabani, Perkerra, Ngambo, Eldume, Sandai, and Maji ndege) were included in the study. The age distribution for the animals sampled was stratified in four categories: ≤1 year, >1–2 years, >2–3 years, >3–4 years and >4 years. From the two subcounties, 10% ($n = 7$) of the sublocations were randomly selected and included in the study. A list of household herds within the seven administrative sublocations was generated in consultation with local administrators, animal health staff and community elders in a baseline visit and used as the sampling frame.

Sample size calculation was based on the formula for sample size determination when herds or other aggregates of animals are the sampling units and taking into account herd effects to achieve high herd level sensitivity and specificity while also accounting for test imperfections as the ELISA kit used had less than 100% sensitivity and specificity (Thrusfield, 2007). We assumed the *C. burnetii* herd seroprevalence was 50% with a desired absolute precision of ±10%. We chose the 50% seroprevalence because it provides the largest sample size for given values of absolute error. Aggregate sensitivity and aggregate specificity prespecified by the investigator was 97% and 94%, respectively. The sample size was determined to be 115 herds, but the final number of herds from which samples were collected was slightly higher (140 herds). In each household, a maximum of six animals of all ages were randomly selected using the lottery method whereby all animals in the herd were numbered using animal marker pens and random numbers assigned by dividing the herd size by six (6) to create the interval of selection. Animals bearing the random number were selected for blood sample collection.

Blood collection and data collection

Blood samples were collected by jugular venipuncture as per the standard operating procedures for animal experimentation (ILRI-IACUC, 2004). A volume of 5 mls of blood was collected into vacutainers and transported in a cool box with frozen ice packs within an average time lag of 6 h from blood collection and delivery to the field laboratory for serum separation. Serum was harvested by centrifugation at 2500 *g* for 10 min. The serum was then collected into cryovials and transported to the Central Veterinary Laboratory in Kabete where it was stored at −20°C until testing. Animal and herd demographic data were collected using standardized household and animal sampling questionnaires (refer to questionnaire supplement). Questionnaires were administered to the household head or his/her representative in interviews immediately after animal sample collection. These questionnaires had been pretested in five households in Marigat during a pilot visit.

Serological analysis

Anti-*C. burnetii* IgG antibodies were detected using a commercially available indirect ELISA kit (LSIVet™ Ruminant Q fever – ELISACOXLS2) following manufacturer's instructions. Briefly, 5 µl of pre-diluted serum samples and controls (positive and negative) were added in the wells of the coated plate and 95 µl of Q fever sample dilution buffer added to each well containing the controls or samples. Each plate was then covered with an adhesive cover plate and incubated for 1 h at 37°C. Three washes were performed with wash solution before 100 µl of conjugate was added and incubated for 1 h at 37°C. The three wash steps were repeated and 100 µl of substrate added to each well and incubated for 10 min at room temperature, and then 100 µl of stop solution was added.

The results were read within 30 min after stopping the reaction at 450 nm on a microplate reader (Biokit, ELX800 ™ - USA). The results were expressed as S/P (sample/positive) ratio calculated as S/P = (OD sample – ODm NC)/(ODm PC – ODm NC). The per cent positivity (PP) was expressed as PP = S/P × 100. Where OD sample is the optical density for the sample, ODmPC is the average optical density for the positive control and ODmNC is the average optical density for the negative control. A serum sample was considered seropositive when the PP value was >40.

Data analysis

Descriptive statistics mainly frequencies and proportions were generated based on the explanatory variables to

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explore their distribution in relation to the outcome. Seroprevalence was estimated as $p = x/n$, where x denoted the total number of animals positive for *C. burnetii* antibodies out of the sample size, n. This formula was used to compute the overall seroprevalence (combined sheep and goats' data) and species-specific seroprevalence by replacing the numerator and denominator to the relevant number of animals in each species.

Univariable mixed-effects logistic regression models were run to assess the relationship between *C. burnetii* antibody seroprevalence and the putative risk factors for *C. burnetii* seropositivity. The putative risk factors assessed included species, breed, sex, age group, parity, lactation, production system and sublocation as the independent variables and *C. burnetii* positivity being the dependent variable. These variables were included in the study based on their biological plausibility and their documented association with the outcome in previous studies. All the independent variables on their own were run against the dependent variable, in a univariable mixed-effects logistic regression model. The results from the univariable mixed-effects logistic regression model including the odds ratio, the 95% CI for the odds ratio and the *P*-value attached to the odds ratio were obtained. The significance level was set at $P \leq 0.2$, and independent variable(s) that met this criterion were included in the multivariable mixed-effects logistic regression model. The variables meeting this criterion were species, breed, sex, age group, parity, production system and sublocation.

A multivariable mixed-effects logistic regression was subsequently built by including significant variables at the univariable mixed-effects logistic regression model. Model building assumed family binomial with logit link functions. The model building strategy employed was a stepwise regression with forward selection from the list of independent variable(s) that had $P \leq 0.2$ at the univariable mixed-effects logistic regression model to find the best fitting model to describe the dataset based on likelihood ratio test ($P \leq 0.05$). Covariates were added one at a time, at each stage including the covariates which resulted in the model with the lowest Akaike information criterion (AIC) value and a significant improvement in the model fit ($P \leq 0.05$). The model with the lowest AIC value was considered the best fitting model. The fitted models were evaluated by including herd as a random effect to adjust for possible clustering of *C. burnetii* seropositivity within herds. Odds ratios (OR) derived from the coefficient estimates from the mixed-effects logistic regression model were used to estimate the strength of correlation between the risk factor and *C. burnetii* sero-positivity. Intracluster correlation coefficient (ρ) describing the degree of similarity among seropositive animals in each household was estimated.

Interactions analysis was performed to test for confounding and effect modification. Only the interactions that increased the model fit, using the Akaike information criterion (AIC), were added to the multivariable mixed-effects logistic regression model described above and were thus used in the model analysis. The interactions included were 'species and breed', and 'production system and breed' with *C. burnetii* seropositivity being the dependent variable at $\alpha = 0.05$. Confounding was monitored by calculating the change in the coefficient of a variable after removing another variable. If the change of the estimates exceeded 25%, the removed variable was considered as a potential confounder.

Species-specific models were also explored among goats only and another restricted to sheep to identify possible differences in risk factors for *C. burnetii* between the two species. The same procedures described above were used to carry out the model building at both the univariable and multivariable analysis stages. However, the interactions analyses were not conducted for the separate species analysis, due to smaller sample sizes. Statistical analysis was performed using R (R DevelopmentCoreTeam, 2015), and the evaluation of the generalized linear mixed-effects models was performed using the *lme4* package in R (Bates et al., 2015).

Ethics and consent statement

The study protocol was reviewed and approved by the International Livestock Research Institute Institutional Research Ethics Committee, (Protocol approval #2014-14). All animal owners provided informed consent before specimen collection and questionnaire administration. The Director of Veterinary Services, Kenya, and Baringo County Director of Veterinary Services also granted permission. The animal restraint and sampling were designed to be less invasive for both animal and personal safety and were conducted by animal technicians and veterinary surgeons according to the World Organization for Animal Health (OIE) guidelines for use of animals in research and education.

Results

Distribution and characteristics of sampled animals, and seropositivity results

Descriptive statistics

A total of 840 animals had samples obtained with 508 (60.5%) goats and 332 (39.5%) sheep being sampled from 140 household herds. The demographic characteristics of sampled animals and seropositivity results varied across species, breed, sex, age, parity, lactation status, production system and administrative sublocation (Table 1). Similar

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Table 1. Demographic characteristics of sampled animals and *C. burnetii* seropositivity results for combined sheep and goats' data and species-specific data

Sheep and goats' data n = 840	Goats n = 508						Sheep n = 332		
Variable	Frequency (%)	Seropositive (n)	% Seroprevalence [95% CI]	Frequency (%)	Seropositive (n)	% Seroprevalence [95% CI]	Frequency (%)	Seropositive (n)	% Seroprevalence [95% CI]
Species									
Goat	508 (60.5)	132	26.0 [22.2, 30.0]	–	–	–	–	–	–
Sheep	332 (39.5)	40	12.2 [8.7, 16.0]	–	–	–	–	–	–
Breed									
Indigenous	723 (86.1)	158	21.9 [18.9, 25.0]	501 (98.6)	130	25.9 [22.2, 30.0]	222 (66.9)	28	12.6 [8.5, 17.7]
Cross-breed	117 (13.9)	14	12.0 [6.7, 19.3]	7 (1.4)	2	28.6 [3.7, 70.6]	110 (33.1)	12	10.9 [5.8, 18.2]
Sex									
Female	680 (80.9)	150	22.1 [19.0, 25.4]	405 (79.7)	114	28.1 [23.8, 32.8]	275 (82.8)	36	13.1 [9.3, 17.7]
Male	160 (19.1)	22	14.0 [8.8, 20.1]	103 (20.3)	18	17.5 [10.7, 20.1]	57 (17.2)	4	7.0 [2.0, 17.0]
Age									
≤1 year	168 (20)	18	10.7 [6.5, 16.4]	125 (24.6)	15	12.0 [6.8, 19.0]	43 (13.0)	3	7.0 [1.5, 19.1]
>1–2 years	236 (28)	49	20.8 [15.8, 26.5]	150 (29.5)	39	26.0 [19.2, 33.8]	86 (25.9)	10	11.6 [5.7, 20.3]
>2–3 years	197 (23.5)	39	19.8 [14.5, 26.0]	94 (18.5)	28	29.8 [20.8, 40.1]	103 (31.0)	11	10.7 [5.4, 18.3]
>3–4 years	119 (14.2)	24	20.2 [13.4, 28.5]	55 (10.8)	20	36.4 [23.8, 50.4]	64 (19.3)	4	6.3 [1.7, 15.2]
>4 years	120 (14.3)	42	35.0 [26.5, 44.2]	84 (16.5)	30	35.7 [25.5, 46.7]	36 (10.8)	12	3.0 [18.6, 51.0]
*Parity									
No parity	135 (19.8)	19	14.1 [8.7, 21.1]	91 (22.5)	17	18.7 [11.3, 28.2]	44 (16)	2	4.5 [0.5, 15.5]
1st parity	118 (17.4)	29	24.6 [17.1, 33.4]	77 (19.0)	23	29.9 [20.0, 41.4]	41 (14.9)	6	14.6 [5.5, 29.2]
2nd parity	123 (18.1)	26	21.1 [14.3, 29.4]	73 (18.0)	20	27.4 [17.6, 39.1]	50 (18.2)	6	12.0 [4.5, 24.3]
3rd parity	115 (16.9)	25	21.7 [14.6, 30.4]	55 (13.6)	18	32.7 [20.7, 46.1]	60 (21.8)	7	11.7 [4.8, 22.6]
4th parity	189 (27.8)	51	27.0 [20.8, 33.9]	109 (26.9)	36	33.0 [24.3, 42.7]	80 (29.1)	15	18.6 [10.9, 29.0]
*Lactation									
Yes	344 (50.6)	78	22.7 [18.4, 27.5]	193 (47.7)	56	29.0 [22.7, 36.0]	151 (54.9)	22	11.3 [6.3, 18.2]
No	336 (49.4)	72	21.4 [17.2, 26.2]	212 (52.3)	58	27.4 [21.5, 33.9]	124 (45.1)	14	14.6 [9.3, 21.2]
Production system									
Mixed farming	300 (35.8)	42	14.0 [10.3, 18.4]	139 (27.4)	25	18.0 [12.0, 25.4]	161 (48.5)	17	10.6 [6.3, 16.4]
Agro-pastoralism	354 (42.1)	59	16.7 [12.9, 21.0]	209 (41.1)	41	19.6 [14.5, 25.7]	145 (43.7)	18	12.4 [7.5, 18.9]
Nomadic pastoralism	186 (22.1)	71	38.2 [31.2, 45.6]	160 (31.5)	66	41.3 [33.5, 49.3]	26 (7.8)	5	19.2 [6.6, 39.4]
Sublocation									
Eldume	102 (12.2)	11	10.8 [5.5, 18.5]	77 (15.1)	10	13.0 [6.4, 22.6]	25 (7.5)	1	4 [1.0, 20.4]

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Table 1. (Continued)

Sheep and goats' data n = 840	Goats n = 508						Sheep n = 332		
Variable	Frequency (%)	Seropositive (n)	% Seroprevalence [95% CI]	Frequency (%)	Seropositive (n)	% Seroprevalence [95% CI]	Frequency (%)	Seropositive (n)	% Seroprevalence [95% CI]
Endao	133 (15.8)	15	11.3 [6.5, 17.9]	81 (15.9)	13	16.0 [8.8, 25.9]	52 (15.6)	2	3.8 [4.7, 13.2]
Maji Ndege	60 (7.1)	11	18.3 [9.5, 30.4]	43 (8.5)	10	23.3 [11.8, 38.6]	17 (5.2)	1	5.9 [1.4, 28.7]
Ngambo	125 (14.9)	17	13.6 [8.1, 20.9]	39 (7.7)	9	23.1 [11.1, 39.3]	86 (25.9)	8	9.3 [4.1, 17.5]
Perkerra	72 (8.6)	16	22.2 [13.3, 33.6]	45 (8.9)	8	17.8 [8.0, 32.1]	27 (8.2)	8	29.6 [13.6, 50.2]
Salabani	186 (22.1)	71	38.2 [31.2, 45.6]	160 (31.5)	66	41.3 [33.5, 49.3]	26 (7.8)	5	19.2 [6.6, 39.4]
Sandai	162 (19.3)	31	19.1 [13.4, 26.0]	63 (12.4)	16	25.4 [15.3, 37.9]	99 (29.8)	15	15.2 [5.7, 23.8]

* Only the female animals had data on parity and lactation collected.

variations were also observed when data were stratified by species (Table 1). The proportion of females was higher compared to males both overall and across the two species (≥80%). Majority of the animals were of indigenous breed (≥70%), while the proportion of animals across the different production systems, age groups and administrative sublocations (the smallest administrative unit in Kenya) was almost similar. Of the 140 household heads interviewed, 15% had introduced a new animal in the herd, while 70% and 72% had found aborted foetus around watering points and grazing fields, respectively. No household had a designated place for lambing or kidding; hence, animals were giving birth in the fields. Majority (67%) reported reproductive disorders in their herds.

C. burnetii antibody seroprevalence distribution

The combined sheep and goat overall *C. burnetii* antibody seropositivity was 20.5% (95% CI: 17.8%, 23.3%). Among the species, goats had a higher *C. burnetii* sero-positivity of 26.0% (95% CI: 22.2%, 30.0%) compared to that of sheep which was estimated to be 12.2% (95% CI: 8.7%, 16.0%) (Table 1). Among the breeds, the indigenous breed had the highest seroprevalence, 21.9% (95% CI: 18.9, 23.3) as compared to cross-breed. Females had the highest seroprevalence, 22.1% (95% CI: 19.0, 25.4). Among the age groups, the youngest (≤1 year), and the oldest age group (>4 years), had the lowest and highest seroprevalence, respectively, namely, 10.7% (95% CI: 6.5, 16.4) and 35.0% (95% CI: 26.5, 44.2). When compared across parities, those on 4th parity had the highest seroprevalence as compared to the other lower parities, 3rd, 2nd, 1st and no parity, 27.0% (95% CI: 20.8, 33.9). Animals that were lactating had a higher seroprevalence when compared to those that were not lactating, 22.7% (95% CI: 18.4, 27.5). Animals raised through omadic pastoralism had the highest levels of seroprevalence when compared to mixed farming and Agro-pastoralism, 38.2% (95% CI: 31.2, 45.6). Among the sublocations in the study, Salabani had the highest seroprevalence, 38.2% (95% CI: 31.2, 45.6). When the data were stratified by species, the seroprevalence varied across breed, sex, age, parity, lactation, production system and administrative sublocation (Table 1).

Univariable risk factor analyses for *C. burnetii* seropositivity

For the univariable analysis using a mixed-effects logistic regression model, species, breed, sex, age group, parity, lactation, production system and sublocation were significantly associated with *C. burnetii* seropositivity at α=0.05, (Table 2). Among the species, goats were 2.6 times likely to

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Table 2. Results of univariable ($P = \leq 0.2$) and multivariable ($P = \leq 0.05$) models assessing relationship between *C. burneti* serostatus and explanatory variables

Combined sheep and goats' data $n = 840$				
Univariable analyses ($P = \leq 0.02$)			Multivariable analyses ($P = \leq 0.05$)	
Variable	Odds ratio[95% CI]	*P*-value	Odds ratio[95% CI]	*P*-value
Species				
Goat	2.6 [1.7, 3.8]	<0.001	2.4 [1.4, 3.9]	<0.001
Sheep	1 [ref.]		1 [ref.]	
Breed				
Indigenous	2.1 [1.1, 4.0]	0.033	1.2 [0.6, 2.3]	0.610
Cross-breed	1 [ref.]		1 [ref.]	
Sex				
Female	1.8 [1.1, 2.9]	0.026	–	
Male	1 [ref.]		–	
Age				
≤1 year	1 [ref.]			
>1–2 years	2.2 [1.2, 4.1]	0.016	1.9 [1.1, 5.1]	0.0170
>2–3 years	2.1 [1.2, 3.7]	0.014	1.7 [1.1, 5.2]	0.037
>3–4 years	2.1 [1.1, 4.2]	0.035	1.5 [1.1, 5.2]	0.022
> 4 years	4.5 [2.3, 8.9]	<0.001	2.4 [1.7, 8.8]	0.0173
*Parity				
No parity	1 [ref.]		1 [ref.]	
1st parity	2.0 [1.0, 4.1]	0.059	1.1 [0.5, 2.6]	0.842
2nd parity	2.3 [1.2, 4.3]	0.012	1.2 [0.4, 3.8]	0.713
3rd parity	1.6 [0.8, 3.4]	0.179	0.9 [0.4, 2.6]	0.985
4th parity	1.7 [0.8, 3.5]	0.145	1.1 [0.4, 3.5]	0.799
*Lactation				
Yes	1.1 [0.7, 1.6]	0.709		
No	1 [ref.]			
Production system				
Mixed farming	1 [ref.]		1 [ref.]	
Agro-pastoralism	1.2 [0.8, 2.0]	0.395	1.1 [0.6, 1.9]	0.794
Nomadic pastoralism	3.8 [2.3, 6.3]	<0.001	3.7 [1.4, 9.1]	0.005
Sublocation				
Eldume	1 [ref.]			
Endao	1.1 [0.4, 2.6]	0.912	1.0 [0.4, 2.5]	0.969
Maji Ndege	1.9 [0.7, 5.0]	0.221	1.5 [0.5, 4.3]	0.462
Ngambo	1.3 [0.5, 3.4]	0.586	1.6 [0.5, 4.8]	0.434
Perkerra	2.4 [0.9, 6.1]	0.078	2.4 [0.9, 6.6]	0.084
Salabani	5.1 [2.3, 11.4]	<0.001	–	–
Sandai	2.0 [0.8, 4.5]	0.115	2.6 [1.1, 6.3]	0.028

*Only the female animals had data on parity and lactation collected.

test positive for *C. burnetii* as compared to sheep, OR 2.6 (95% CI: 1.7, 3.8); $P < 0.001$. Indigenous breeds were 2.1 times likely to be positive for *C. burnetii* when compared to cross-breeds, OR 2.1 (95% CI: 1.1, 4.0); $P = 0.033$. Females were 80% more likely to be seropositive, OR 1.8 (95% CI: 1.1, 2.9); $P = 0.026$. When testing positive for *C. burnetii*, all the older age groups had higher odds when compared to the youngest age group with the oldest age group being 4.5 times likely to test positive, OR: 4.5 (2.3, 8.9); $P < 0.001$. Among the parities when compared to those with zero parity, those on 2nd parity had the highest odds of being seropositive, they were 2.3 times likely to be positive for *C. burnetii*, OR 2.3 (95% CI: 1.2, 4.3); $P = 0.012$. Animals that were lactating were 10% more likely to be seropositive when compared to those not lactating, although the results are not statistically significant, OR 1.1 (95% CI: 0.7, 1.6); $P = 0.709$. Nomadic pastoralism production system had the highest odds of animal contracting *C. burnetii* when compared to mixed farming, OR 3.8 (95% CI: 2.3, 6.3); $P < 0.001$. When the sublocations in the study were compared to Eldume as the reference category, Salabani

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had the highest odds among the other sublocations of testing positive for *C. burnetii* when compared to Eldume sublocation, OR 5.1 (95% CI: 2.3, 11.4); $P < 0.001$ (Table 2).

Multivariable risk factor analyses for *C. burnetii* seropositivity

Mixed-effects multivariable logistic regression model returned species, age group and nomadic pastoralism as factors correlated to *C. burnetii* positivity at α=0. 05 (Table 2). Goats were 2.4 times likely to test positive for *C. burnetii* when compared with sheep, OR 2.4 (95% CI: 1.4, 3.9); $P < 0.001$. Indigenous breeds were 20% more likely to be seropositive as compared to cross-breeds although this result is not statistically significant, OR 1.2 (95% CI: 0.6, 2.3); $P = 0.610$. Among the age groups when the older age groups were compared with the youngest age group (≤1 year), the odds were higher with the highest odds being attributed to the oldest age group (>4 years), OR 2.4 (95% CI: 1.7, 8.8); $P = 0.0173$. Animals in nomadic pastoralism had significantly higher odds of testing positive for *C. burnetii* when compared to those in mixed farming; they were 3.7 times likely to test positive for *C. burnetii*, OR 3.7 (95% CI: 1.4, 9.1); $P = 0.005$. Among the sublocations, when compared to Eldume as the reference, all the sublocations had significantly higher odds of testing positive for *C. burnetii*, with Sandai having odds 2.6 times those of Eldume of testing positive, OR 2.6 (95% CI: 1.1, 6.3); $P = 0.028$ (Table 2). The Intracluster correlation coefficient (ρ) was 0.25.

For the mixed-effects multivariable logistic regression model including interactions, we tested interactions between 'species and breed' and 'production system and breed'. However, none of the interactions were statistically significant.

Species-specific univariable and multivariable models

There were no different risk factors revealed when species-specific univariable and multivariable mixed-effects logistic regression models were explored including breed, sex, age group, parity, lactation, production system and sublocation with *C. burnetii* seropositivity being the dependent variable at α=0.05, (Table S3) (refer to table supplement).

Discussion

Here, we report a high prevalence of *C. burnetii* antibodies in small ruminants, in a population that had previously reported episodes of acute Q fever in humans. Further, we identified factors associated with greater risk of *C. burnetii* seropositivity and explored the implications of the results to disease transmission, burden and its control. Our results demonstrated that *C. burnetii* has been circulating in the small ruminant population in Baringo County region of Kenya.

In this study in rural Baringo, 26.0% of goats and 12.2% of sheep had antibodies to *C. burnetii*. Mixed-effects multivariable logistic regression analysis indicated that seroprevalence varies by species, age and the type of livestock production system. Goats had higher seropositivity compared to sheep, while older animals were more likely to be seropositive compared to young animals (≤1 year). Nomadic pastoralism production system had the highest odds of animals being *C. burnetii* seropositive when compared to other livestock production systems. *C. burnetii* seroprevalence was heterogeneous across the sublocations with animals in Sandai being 2.6 times likely to be seropositive when compared to Eldume.

Although our estimated seroprevalence is slightly lower than the 32% in goats and 18.2% in sheep detected in a recent study in rural western Kenya, the studies showed a similar trend where goats are significantly correlated with *C. burnetii* seropositivity compared to sheep (Knobel et al., 2013). A critical global literature review on the prevalence of *C. burnetii* infection in domestic ruminants in several countries showed a wide variation in reported prevalence and the quality of the studies contacted (Guatteo et al., 2011). The review estimated average prevalence on animal level as 15% and 27% for sheep and goats, respectively (Guatteo et al., 2011). However, of the 69 published papers reviewed, only four studies on domestic small ruminants were carried out in Africa, which highlights the significant gap in *C. burnetii* burden and risk analysis studies in Africa despite *C. burnetii* being highly endemic globally (Guatteo et al., 2011). In Chad, Central Africa, a region with almost similar production system to our study area, seroprevalence of 11% and 13% was found in sheep and goats, respectively (Schelling et al., 2003). Results of our study showed a higher seroprevalence among small ruminants, which implies that *C. burnetii* is of considerable importance in the small ruminant population of Kenya.

Despite the study's limitation of relying on the animal owners' responses to ascertain the animal age (due to lack of proper animal records and the logistical challenge of animal ageing by dentition), age was found to be significantly correlated with *C. burnetii* seropositivity with older animals being more likely to be seropositive compared to young animals. The difference was more pronounced when animals less than 1 year were compared to animals greater than 4 years, where animals >4 years were almost three times more likely to be exposed compared to animals less than 1 year. The results are consistent with previous studies that have shown the likelihood of seropositivity increases

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with age, with peak prevalence being achieved at reproductively mature animals at 2–3 years (Knobel et al., 2013; Van den Brom et al., 2013a; Ylli et al., 2013). This is attributed to the horizontal nature of *C. burnetii* transmission and the animals' exposure to the pathogen continuously from a young age, due to the predominant practice of the livestock production system in this setting in which they are allowed to freely roam during grazing which increases the frequency of contact between herds.

Goats had 2-fold higher likelihood of exposure to infection compared to sheep. Varying seroprevalence in sheep and goats has been documented with some studies documenting higher seroprevalence in goats (Knobel et al., 2013; Klaasen et al., 2014), while others recording higher seroprevalence in sheep (Ruiz-Fons et al., 2010; Ylli et al., 2013). The difference in inherent susceptibility to *C. burnetii* between sheep and goats has not been previously demonstrated, and hence, this needs further research.

Animals reared in a nomadic pastoralism system had three times higher likelihood of being *C. burnetii* seropositive compared to animals in other systems. This could be attributed to the extensive mobility and contact between different pastoral herds during grazing and aggregation in common watering points, compounded by the fact that *C. burnetii* persists and remains infectious in the environment for long periods (Raoult et al., 2005). The result is consistent with other previous studies which showed that sedentary systems of livestock rearing were associated with reduced odds of *C. burnetii* seropositivity while grazing systems and systems that allow for more random contact between uninfected and infected animals are associated with increased odds of *C. burnetii* antibody seropositivity (Taurel et al., 2011; Alvarez et al., 2012; Paul et al., 2012). Data from the questionnaire survey indicated evidence of introduction of new animals in the herds, animals giving birth in the fields, presence of aborted foetuses in grazing fields and a round watering points as well as reproductive disorders in majority of the herds. These are potential routes of *C. burnetii* exposure both in livestock and humans which puts these populations at higher risk.

Although a direct link between the human *C. burnetii* cases previously reported in the study region, and the *C. burnetii* seropositive domestic small ruminants in the current study could not be established, there is a plausible zoonotic public health risk link of animal to human transmission which may need further investigation (Chang et al., 2010; Dean et al., 2013; De Lange et al., 2014; Bond et al., 2015).

Coxiella burnetii is highly infectious with 1 –10 viable organisms being able to cause infection in humans (Sawyer et al., 1987; Gurtler et al., 2014) and persists in the environment for long periods, increasing the risk of exposure in people living in *C. burnetii* prevalent areas either through exposure to contaminated environmental reservoirs such as manure and soil, or contact with infected animals (Amitai et al., 2010; Smit et al., 2012; Kersh et al., 2013; Gurtler et al., 2014).

Interpretation and generalization of these results should bear in mind design, sampling, sample size, study population and diagnostic limitations. In this study, an indirect IgG ELISA was used to detect the presence of antibodies against *C. burnetii*. The choice of serology as the test method is limited because the presence of antibodies may not be evidence of active *C. burnetii* infection but only shows an animal has been previously exposed to *C. burnetii* (Sidi-Boumedine et al., 2010; Muskens et al., 2011). This limits the interpretation of the results at an individual animal level as the presence of these antibodies indicates past exposure and may not be evidence of current infection or actual shedding of bacteria. This can be attributed to animals being able to seroconvert without detectable shedding as well as their ability to remain seropositive for a long time after infection has resolved while other animals shed bacteria but never seroconvert (McQuiston et al., 2002). However, serology is a suitable and a cost-effective technique for screening herds (EFSA, 2010) as it provides insights on the level of exposure to the pathogen in the region.

The study utilized a cross-sectional study design which may not be entirely suitable for investigation of putative risk factors of the pathogen as it does not allow the investigator to determine the time of first infection or introduction of the pathogen to the herd and hence may pose a danger of misclassification of the risk factors associated with seropositivity. However, the aim of our study was to identify associations and not cause–effect relationships; hence, the design was considered sufficient to identify the factors with significant associations to the study outcome.

Sample size was estimated for a combined sheep and goats' data which limited our stratified analyses. A large sample would have been needed on both strata to carter for the extra stratified analysis; this would have been achieved through multistage sampling with stratified sampling at stage one.

Our study focused on evidence of *C. burnetii* exposure in small ruminants. However, other livestock such as cattle as well as humans are also susceptible. Further studies in this area should incorporate cattle and humans in a linked human – livestock study to provide a more comprehensive picture of the disease epidemiology.

Data on environmental factors such as precipitation, soil types and vegetation cover were not collected. These would have been beneficial in explaining the heterogeneity in *C. burnetii* seroprevalence across the sublocations.

Although the use of a fixed number of animals per herd and the random selection of animals might have led to

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missing of positive animals in case of low prevalence herds, the present study established the presence of *C. burnetii* antibodies in domestic small ruminants, an indication the pathogen is circulating in this setting.

In conclusion, our findings suggest high levels of *C. burnetii* exposure in small ruminants, a potential risk of exposure to humans in this setting. These insights on the circulation of *C. burnetii* among the study populations provide baseline information which can be incorporated when planning and designing prevention and control strategies against the *C. burnetii* in this setting. Such control measures should include improving biosecurity measures in livestock management system, surveillance and laboratory tests before purchase of animals to detect putative *C. burnetii* shedders and restricted movements between herds.

Finally given the uncontrollable airborne *C. burnetii* spread between farms, compounded by the open landscapes with very little vegetation and little precipitation, vaccination may be the only adequate control measure to implement in this setting. Animal vaccination will also significantly reduce the disease incidence in humans by controlling the disease at the reservoir.

Further studies to identify the presence of active infection using direct diagnostic techniques are needed in order to evaluate the true risk posed by small ruminants as sources of *C. burnetii* infection are needed. To address the risk factors for *C. burnetii* transmission between animals and humans, we recommend integrated animal–human surveillance and socio-economic studies for *C. burnetii*. Such studies should inform public health measures aimed at mitigating the risk of transmission in both domestic ruminants and humans, reducing the impact of the disease in these populations.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

JM, SK, SMT, MO, AB, EO and MN conceived and designed the study. JM collected and cleaned the data. JM and SW performed the experiments. JM, SMT, MO and MN analysed the data. JM drafted the manuscript. All authors helped with the interpretation of the results, read, critically reviewed and approved the final manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Animal Factor Data Collection and Sample Tracking Tool.

Data S2. Household questionnaire.

Table S3. Results of species specific univariable ($P = \leq 0.2$) and multivariable ($P = \leq 0.05$) models assessing relationship between *C. burnetii* sero-status and explanatory variables.

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