MOLECULAR DETECTION AND RISK FACTORS ANALYSIS OF COXIELLA BURNETII AND CHLAMYDIA ABORTUS INFECTIONS IN SHEEP AND GOATS IN KAJIADO, KENYA.

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF VETERINARY PUBLIC HEALTH.

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November, 2021

DECLARATION

This thesis is my own original work and has not been submitted for an award of degree in any other university.

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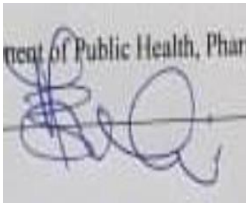This thesis has been submitted for examination with our approval as university supervisors

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COXIELLA BURNETII, CHLAMYDIA ABORTUS INFECTIONS IN SHEEP AND GOATS

IN KAJIADO, KENYA

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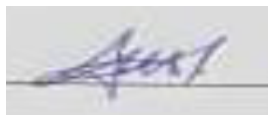
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DEDICATION:

I wish to dedicate this thesis to my father Ibrahim, brothers, sisters, my lovely husband Abdideeq daughter Bushra and my son Abdillaahi. It was through your prayers, unconditional love, endless inspiration, and support that I have made it this far.

ACKNOWLEDGEMENT:

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LIST OFABBREVIATION:

BBSRC - Biotechnology and biological sciences research council

BLAST - Basic local alignment search tool

bp - Base pair

C. abortus - *Chlamydia abortus*

C. burnetii - *Coxiella burnetii*

CFT - Complement fixation test

DNA - Deoxyribonucleic acid

dNTP - Deoxynucleotide triphosphate

EB - Elementary body

EDTA - Ethylene diamine tetra acetic acid

ELISA - Enzyme linked immuno-sorbent assay

ICT - Immuno-chromatographic test

IFT - Immunofluorescence tests

ILRI - International livestock research institute

NAA - Nucleic acid amplification-based tests

NACOSTI - National Commission for Science, Technology and Innovation

ODK - Open data kit

OIE - Organization for Animal Health

OR - Odd's ratio

PCR - Polymerase chain reaction

PHPT - Public Health Pharmacology and Toxicology

SPSS - Statistical Package for Social Sciences

RNA - Ribonucleic acid

RB - Reticulate body

WB1 - Wash buffer 1

WB 2 - Wash buffer 2

ABSTRACT:

Chlamydia abortus (*C. abortus*) infection and Q fever caused by *Coxiella burnetii (C. brunetii)* are zoonotic diseases caused by obligate intracellular bacteria. The infections cause economic losses in sheep and goat production systems in many parts of the world. The diseases are also of public health importance since they can infect humans. Information on the status of the two infections and risk factors responsible for the outbreak infections in sheep and goats is limited or lacking in Kenya, especially in pastoral communities. Moreover, molecular detection for confirming infections in shoats has not been exploited widely in Kenya. As a consequence, the objective of this study was to investigate into the presence of Q-fever and *C. abortus* infection in sheep and goats in five selected wards in Kajiado County. One hundred and thirty pastoralist flocks were selected from the five wards, which included Ildamat (27 flocks), Iloodokilani (27 flocks), Matapato south (25 flocks), Kenyawa-Poka (21 flocks), and Kaputiei north (30 flocks). After that, 1560 sheep and goat blood samples were collected from these flocks in the five wards. The samples were then transported to the Department of Public health, Pharmacology Laboratory for further analysis. The genomic DNA was extracted from whole blood samples using the Gene JET commercial Mini-Kit according to the manufacturer's instructions. Oligonucleotide primer targeting IS1111transposase element of *C. brunetti* and 16S-23S rRNA of *C. abortus* was used to amplify the DNAs by polymerase chain reaction (PCR) using the Veriti 96 well thermos-cycler. The amplicons were electrophoresed, stained, and visualized by a gel documentation imager. To assess the risk factors as well as to establish knowledge, attitudes, and practices of the pastoralist farmers, questionnaires were administered to respondents responsible for the flock. The prevalence of the two infections was estimated, and risk factors were determined by logistic regression. *Coxiella burnetti*-DNA was not detected in all samples analyzed. *Chlamydia*

abortus DNA was detected in 86 (24.8%) sheep and goats blood samples, with 30(20.3%) samples being detected in sheep while 56 (28.1%) samples were detected in goats. Although samples positive for *C. abortus*-DNA were more in goats than those in sheep, the difference observed was not statistically significant (P <0.0.5). The prevalence of *C. abortus* in the five wards was 34.96% in Ildamat, 31.6% in Iloodokilani, 10.9% in Mathapato south 15.4% in Kenyawa poka, and 18.4% in Kaputiei. Approximately 56% of the farmers reported abortions as the main problem in their flocks. They believed that infections such as Brucellosis, Rift valley fever, tick-borne diseases were the major problems in their flocks. The study also found that 27.7% of the respondents were aware of zoonotic diseases. Furthermore, it was also observed that 39 (30%) farmers were aware that some of these diseases could also be transmitted to humans through the consumption of milk and meat. The risk factors associated with *C. abortus* infection were the watering points for animals during the wet season (OR=1.57, P=0.02), abortions (OR.84, P=0.02), and consumption of fermented raw milk by household pastoralists (OR=1.25, P= 0.05). In conclusion, it appears that sheep and goats raised in Kajiado County are infected with *C. abortus*. Transmission of *C. abortus* may be enhanced by overcrowding of sheep and goats at a drinking point during the wet season, as well as abortion in flocks. Further research on detection and risk factors of the two infections among pastoralists is recommended.

CHAPTER ONE: INTRODUCTION

1.1. Background information:

Chlamydia abortus (*C. abortus*) infection and Q fever caused by *Coxiella burnetii (C. brunetii)* are important obligate intracellular bacterial infections of livestock and humans that cause clinical conditions resulting in infertility and production loss. The diseases are clinically and epidemiologically significant worldwide, both in humans and animals (Rohde *et al.,* 2010). *Chlamydia abortus* is a gram-negative, intracellular obligate bacterium that infects sheep and goats' mucosa (Cheong *et al.,* 2019). Chlamydia is distributed throughout the world, causing various diseases in both humans and animals (Szymańska-Czerwińska *et al.,* 2017). *Chlamydia abortus* is zoonotic disease. Although most human infections are mild and often unnoticed, pregnant women can develop severe, life threatening illnesses and abortions (Nietfeld, 2001).

Chlamydia abortus is transmitted through aborted products such as fetus and placenta. The other mode of transmission includes oral route and inhalation of dust particles by the susceptible animals (Bagley, 2001). In many countries, it is one of the causes of abortion and fetal loss in sheep and goats (Li *et al.,* 2015). Subsequently, the disease causes a significant negative impact on the livestock industry in many countries worldwide (Cheong *et al.,* 2019). *Chlamydia abortus* infection causes serious economic losses in both animal production performance and public health. The cost is as result of high prevalence of the disease and high costs of diagnosis, vaccination, treatment, and management (Postma *et al,* 2002).

Q-fever is a bacterial infection that causes a variety of clinical symptoms in livestock, including infertility and production loss (Njeru *et al.,* 2016). All domesticated ruminants are susceptible with the cases of reproductive failures such as abortions, stillbirths, and infertility being reported.

Typically, Q-fever infection is asymptomatic, and the animals can remain infected for a long time without showing clinical signs (Scolamacchia et al., 2010). Infection in humans usually occurs via inhalation of contaminated aerosols (Klemmer et al., 2018). Q-fever usually manifests as nonspecific symptoms such as high fever of up to 41^0C, severe headache, fatigue, chills, cough nausea, vomiting and diarrhea. These nonspecific symptoms can progress to severe chronic disease and in most cases they can be misdiagnosed (Scolamacchia et al., 2010). The economic and public health impacts of Q fever remain a major concern in developing countries because Q fever causes significant loss of animal productivity (Tagesu, 2019). The disease causes heavy economic losses in flocks due to abortions and birth of weak offspring (Eibach et al., 2012).

Due to abortions and the birth of weak offspring, the disease causes significant economic losses to pastoralists. Nevertheless, in some situations, the animals can recover without complications (Eibach et al., 2012). Coxiella burnetii infection, can last for years, and domestic ruminants are often subclinical carriers, the hosts can shed the bacteria in various excretions such as urine, milk, faeces, placental and birth fluids (Abbas et al., 2011). A seroprevalence of 54.2% for C. burnetii has been reported for goats reared in the Somali and Oromia regional states of southern Ethiopia (Tagesu, 2019). In Kenya, the seroprevalence of C. burnetii in sheep and goats has been reported to be 57.5% and 83.1% respectively (Njeru et al., 2016) .

Detection of Q-fever and C. abortus infections in sheep and goats is critical for effective control of the two diseases (Barkallah, et al., 2018). Subsequently, some studies have documented the use of molecular techniques such as PCR for the detection of the two pathogens. Other studies have also focused on detection of circulating antibodies in order to assess the level of exposure to the bacterial infection (Jung et al., 2014). The most effective way to reduce the impact of these pathogen infections on flock health and productivity is to improve the ability to monitor and avoid

pathogen transmission. Therefore, small ruminant management and environmental factors that increase the risk of infection must be taken into account. In this regard, it has been also possible to assess the risk factors associated with outbreaks of Q-fever and *C. abortus* infections in sheep and goats (Talafha *et al*. 2009). These risk factors include awareness of farmers, abortions, raising the flocks in crowded conditions among others (Merdja *et al.,* 2015). In this study, *C. abortus* and *C. brunetii* were detected by molecular analysis using PCR. The risk factors responsible for the outbreaks of the two diseases were also assessed in Kajiado County in Kenya.

1.2. Problem statement:

Chlamydia abortus is one of the most common causes of reproductive losses in sheep and goats globally, except in Australia and New Zealand, which are disease-free. In many sheep-rearing areas worldwide, *Chlamydial* abortion in late pregnancy causes major economic loss, mainly where flocks are densely concentrated during the kidding and lambing season (Longbottom, 2008). Being zoonotic disease, *C. abortus is* also a public health priority disease (Selim, 2016). In Kenya, an antibody against *C. abortus* has been previously detected in sea sheep (Wandera, J. G. *et al.,* 1971). In addition, the state of abortions and reproductive defects in livestock, such as premature birth, death and weak offspring, have been linked to Q fever. Q fever is a serious zoonosis because it is extremely infectious in humans and can be a risk to veterinarians, laboratory employees and abattoir workers (Anderson *et al.,* 2013). A recent serological study has revealed that the overall prevalence of *Q fever* in sheep and goats in Kajiado is 57.5% and 83.1%, respectively (Njeru *et al*., 2016).

Chlamydia abortus and *Coxiella burnetii* are known to infect sheep and goats in many regions of the world except in Australia and New Zealand. The current information on the infections of sheep and goats with the two pathogens is either limited or lacking in Kenya. Furthermore, molecular

detection of *C. abortus* and *C. burnetii* infecting sheep and goats in Kenya has never been exploited yet. This detection technique could be useful in confirmation of active infections. There is also a lack of updated information on the risk factors responsible for the outbreak for the two infections. Therefore, this study determined the concurrence and associated risk factors of Chlamydia infection and Q-fever in sheep and goats in select areas in Kajiado County, Kenya.

1.3. Justification:

Livestock plays an essential role in the livelihood of the majority of population in Kajaido County. Pastoralism is a significant economic activity in the County, with the crucial stocks being cattle, sheep, and goats. Small ruminants are ranked high in importance since they are a source of regular cash income and insurance against tragedies. *Chlamydia abortus* and *Coxiella burnetii* are the most common infectious causes of abortion and the birth of weak lambs in many small ruminant-rearing countries. The abortions are common in the last two to three weeks of pregnancy (Barati *et al*., 2017). These infections are under-reported and under-diagnosed, mainly because the symptoms are mostly non-specific, making diagnosis difficult *(*Anderson *et al.,* 2013).

Therefore, this research has focused on the identification of *C. abortus* and *C. burnetii* from sheep and goats in Kajaido County in Kenya. Subsequently, this study has provided preliminary data on molecular identification of *C. abortus* and *C. burnetii* and the risk factors responsible the transmission and outbreaks of Q-fever and *C. abortus* infection. In this regard, the information generated here can assist with strategies for controlling and preventing the infections in animals and humans. If these diseases are controlled and prevented, then the production losses associated with outbreaks of the two diseases will be minimized. This will eventually ensure increased production of sheep and goats thereby enhancing food security, improving public health and livelihoods among pastoralists community.

1.4. Hypothesis of research:

- Sheep and goats reared in Kajiado County are not infected with *C. abortus* and *C. burnetii*.

- There are no risk factors associated with *C.abortus* and *C. burnetii* infections in small ruminants in Kajiado County.

1.5. Objectives:

1.5.1. General objective:

To assess the occurrence and evaluate risk factors of *C. abortus* and *C. burnetii* infection in small ruminants in Kajiado

1.5.2. Specific objectives:

1. To identify C. *burnetii* and *C. abortus* infecting sheep and goats in selected areas in Kajiado County, Kenya.

2. To analyze risk factors associated with *C. abortus* and *C. burnetii* infections in sheep and goats in Kajaido County, Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Etiology of *Chlamydia abortus* and *Coxeilla burnetii*:

2.1.1 Etiology of *Chlamydia abortus:*

Chlamydia abortus is a common obligate intracellular bacterial infection of livestock and humans that causes a variety of clinical symptoms, including infertility and production loss. The diseases are clinically and epidemiologically significant in both humans and animals around the world (Parker *et al.*, 2006). *Chlamydophila spp.* has been reported in sheep, pigs, goats, koalas, dogs, rabbits, ferrets, and opossums, among many other species (Rodolakis *et al.,* 2010).

Chlamydia abortus is an obligate intracellular bacterium, which infects eukaryotic cells. They have two distinct morphological forms: Elementary body (EB) and reticulate body (RB), and they have a distinct developmental period. Elementary bodies have a circular form and a diameter of 0.2-0.6 m, while RBs have a larger diameter and a more amoeboid shape, with a diameter of up to 1.5 m. Infections with *C. abortus* cause a wide range of clinical diseases in humans and animals. (Everett *et al.,* 1999).

2.1.2 Etiology of *Coxiella burnetii.*

Coxiella burnetii is a gram-negative bacterium that is obligate intracellular and has a diameter of 0.2–1.0m. *Coxiella burnetii* has very high infectivity in humans with a single viable *C. burnetii* being able to cause infection in humans (Brooke *et al.*, 2013). The bacterium exhibits characteristic antigenic variation where it can undergo sporulation making it resistant to adverse environmental conditions making it difficult to disseminate. *Coxiella burnetii* has been isolated from blood, lungs, spleen, liver, urine, feces, milk and placenta of infected humans and animals. (Ta *et al*., 2008). Therefore, isolation of *C. burnetii* from these organs and tissues indicate the importance of cell culture in the characteristics of this gram-negative bacterium. This gram-negative bacterium has

inner and outer membranes, which are different. Prions fused to the outer membrane compensate for the lack of an energy source. Unlike Gram-positive bacteria, it lacks a strong peptidoglycan cell wall. The periplasmic space is the space between the two membranes. Membrane anchors produce lipopolysaccharides. *Coxiella burnetii* bacteria enter the life cycle of the host cell through phagocytosis, where they live in phagocytic vacuoles and multiply in the phage lysozyme (Seshadri *et al.,* 2003).

2.2 Epidemiology of *Chlamydia abortus* and *Coxeilla buretii:*

2.2.1. Epidemiology of *Chlamydia abortus*

Chlamydial infections have been reported in almost all continents. Animal chlamydial infections have been recorded in 22% of countries around the world. When it comes to actual occurrence, this number is most likely greatly underestimated. The prevalence of chlamydial infections in animals is usually high. The disease is commonly misdiagnosed because the length history of the disease, the intracellular nature of the pathogen and limited detection technologies with high sensitivity and specificity (Forsbach-Birk, 2013).

Human *Chlamydial* infections, primarily caused by *C. trachomatis*, are linked to sexually transmitted diseases and blindness in 73 % of all countries (Forsbach-Birk, 2013). Generally, flocks become infected after asymptomatic carrier animals with chlamydial infection are introduced. Ingestion of *C. abortus*, which is excreted in diseased placentas, uterine discharges, and faeces by infected aborting ewes, can induces infection in uninfected sheep at lambing time. The infection in livestock remains asymptomatic until the last 4 weeks of pregnancy. The highest epidemic of this illness in a flock usually occurs in the second lambing season after contracting the *C. abortus* infection. *Chlamydia abortus* infection has been reported in sheep and goats where

they cause abortions in a large number of ewes and does during a single lambing season (Rodolakis *et al*., 2010).

2.2.2. Epidemiology of *Coxiella burnetii*

After an outbreak of febrile sickness among abattoir staff in Queensland, Australia, in 1935, Q fever was described as a zoonotic disease. Since the disease's etiopathogenesis of the disease was unknown, it was given the name "Query (Q)" fever (Mcquiston *et al.,* 2002). Many vertebrate species can be infected, although cattle, sheep, and goats are considered the primary reservoirs. (Anderson *et al.,* 2009). The disease is mostly a professional 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 handling infected animals (Anderson *et al.,* 2013). Wild animals, domestic animals, birds, and arthropods, mostly ticks, have all been identified as reservoirs for *C. burnetii.* In most published investigations, domestic ruminants have been confirmed as the most incriminating source of *C. burnetii* infection in humans. The disease in animals is mostly asymptomatic and in most cases proceeds to the chronic phase. Environmental contamination with *C. burnetii* occurs mostly during parturition when a lot of *C. burnetii* pathogens are shed into the environment (Mcquiston *et al.,* 2002).

2.3 Lifecycle of *Chlamydia abortus* and *Coxiella burnetii*

2.3.1   Lifecycle of *Chlamydia abortus*.

The order Chlamydial bacteria are a diverse group of bacteria that need to develop in eukaryotic cells in order to survive. The elementary body and the reticulate body are the two morphological types of *Chlamydia*. elementary bodies are metabolically inactive extracellular forms that propagate infection by attaching to and entering susceptible cells. elementary bodies have internalized in membrane-bound vacuoles known as inclusions after infection. elementary bodies

differentiate into metabolically active RBs, which go through repeating binary fission cycles, before reverting to EBs. As the host cell lyses, EBs are released into the surrounding cells. Under stressful growth conditions imposed by immune responses, antibiotics, or food shortage, the developmental cycle is interrupted, resulting in wide, abnormal RBs. The altered growth scheme appears to be linked to the continuous expression of genes involved in DNA replication, but not those involved in bacterial cell division (AbdelRahman *et al.,* 2005).
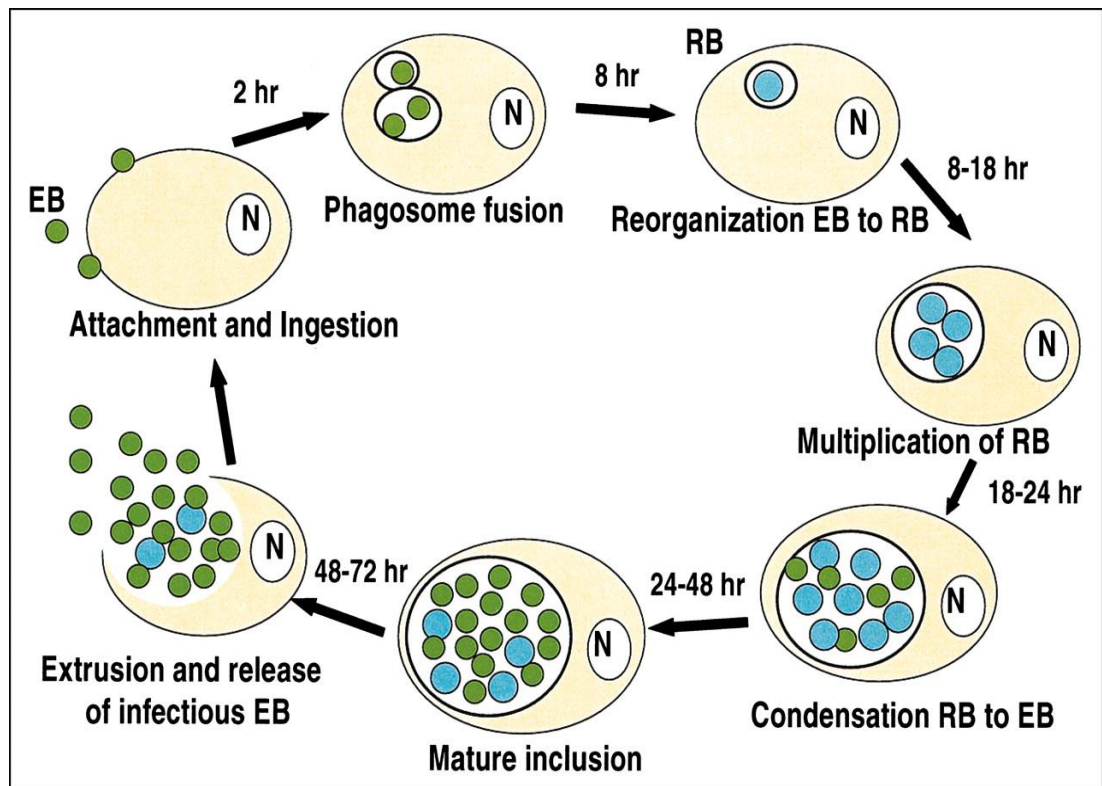
Figure 1: Lifecycle of *C. abortus* showing the different stages of the bacterium during the 6-8days of the lifecycle.

2.3.2 Lifecycle of *Coxiella burnetii.*

Coxiella burnetii is gram-negative intracellular bacteria that completes its life cycle in the phagolysosome of eukaryotic cells. On transmission electron microscopy examination *C. burnetii* appear as pleomorphic organism with two distinct morphological cell types such a large cell variation (LCV) and a small cell variant (SCV). Furthermore, the internal structure of *C. burnetii* cells stained with potassium permanganate show various variants in their internal structure of the cell types.

The underlying dense layer of the SCV, on the other hand, are much wider and more visible than that of the LCV. The SCV's periplasmic space is difficult to see, while the LCV's periplasmic space is visible and appears to be similar to that of other gram-negative bacteria. The cytoplasmic membrane appears to be the source of complicated internal membranous invasions in the SCV. The LCV's membranous system is underdeveloped. Some LCVs have a thick bulk in their periplasmic area (McCaul and Williams, 1981). This endogenous structure appears to start as an electron-dense "cap" at one of the LCV's poles, followed by the steady development of a dense body measuring 130 to 170 nm in diameter. This dense body is eventually encircled by at least four layers of coat. The morphogenesis of *C. burnetii* is similar to, but not identical to, the formation of endospores and cellular differentiation (Seshadri *et al.,* 2003).
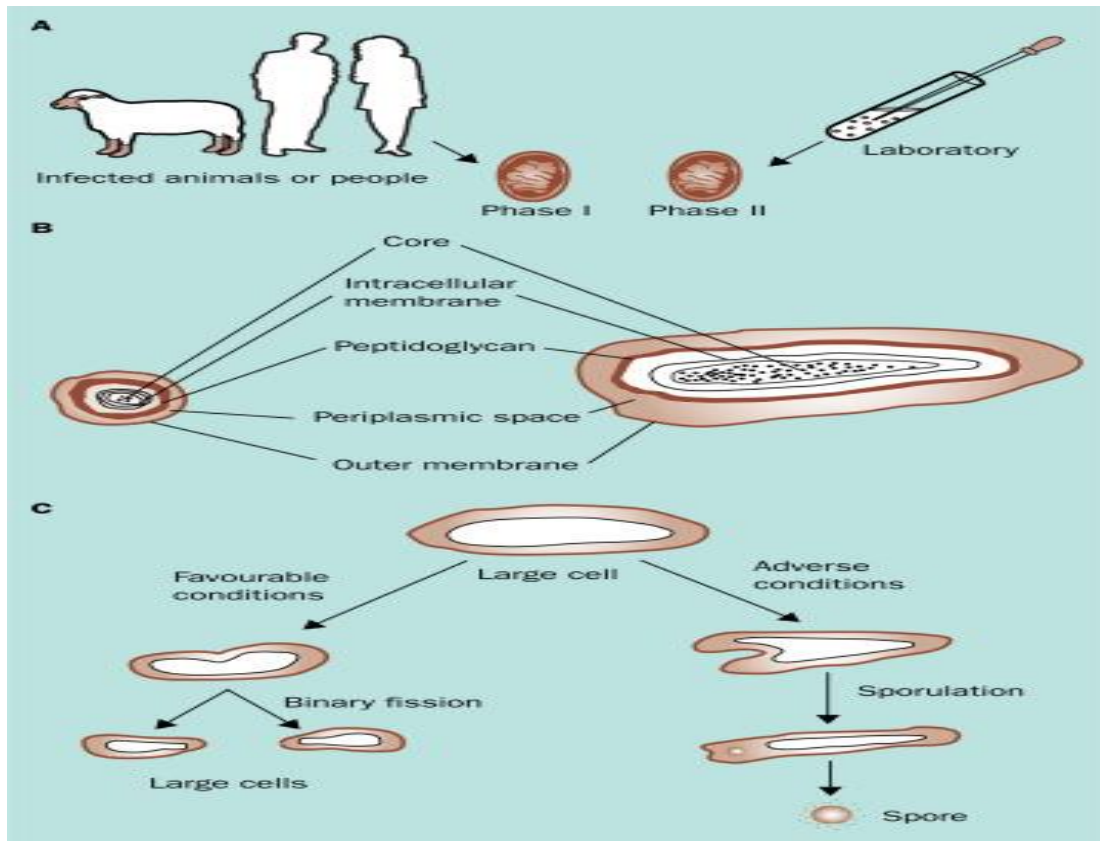
Figure 2: Lifecycle of *C. burnetii* showing the different stages of the bacterium during the 14 days of the lifecycle.

2.4 Transmission of *Chlamydia* and *Coxiella* to human

2.4.1 Transmission of *Chlamydia* abortus to human:

Humans can contract *Chlamydia* if they are exposed to birth fluids and placentas of infected animals. Infection in humans has been reported after contact with infected sheep and goats. (Villemonteix *et al.,* 1990). Small ruminants contaminated with *C. abortus* strains pose a significant risk to expectant mothers. In addition, non-pregnant women can contract *Chlamydial* respiratory disease after inhaling contaminated material from sheep and goats (Keeling *et al.,*1986).

2.4.2. Transmission of *Coxiella burnetii* to human:

The main mode of transmission of *C. burnetii* infection from infected animals to humans is by inhalation of infected aerosols from infected animal reservoirs (Porter *et al.,*2011). Although uncommon, the disease has been confirmed to spread through ingestion, primarily through the consumption of infected dairy products. (Raoult *et al.,* 2005). Infected animals can include cattle, pigs, goats, dogs, and cats, and these animals serve as a source of infection to humans. Infected animals excrete significant quantities of this pathogenic bacterium in their urine, feces, and milk, and these which can be inhaled, ingested, or come into contact with humans. The bacterium may have been isolated from the placenta and uterus of infected animals. Domestic and livestock animals are not the only ones who are affected (Eldin *et al.,* 2017).

2.5. Transmission of *Chlamydia* and *Coxiella* in animals.

2.5.1. Transmission of *Chlamydia abortus* in animals.

co-infections, secondary infections, malnutrition, immunological state, and other stressors, such as transportation and overcrowding may play a role during the transmission and development of *C. abortus*. *Chlamaydia abortus* is commonly transmitted to other species by birth fluids, but it can also be found in other secretions and excretions like faeces. When pregnant ruminants abort or give birth, they may shed significant quantities of *C. abortus* in the placenta and vaginal fluids. Shedding in vaginal fluids can start more than two weeks before an abortion, and it can last for a few weeks afterward, particularly in goats. Sheep and goats may become carriers of *C. abortus,* and some sheep have been infected for at least 2-3 years. These animals can shed the organism around the time of oestrus and during subsequent pregnancies, although recent studies indicate that the quantities are small (Lukac *et al.,* 2017).

2.5.2. Transmission of *Coxiella burnetii* in animals.

The pathogen *C. burnetii* causes Q fever, which affects people all around the world. Due to a lack of symptoms and a low infective dosage of *C. burnetii*, the condition might go misdiagnosed and untreated before causing serious health problems. Infected aerosols of *C. burnetii* can be transmitted by direct contact, but aerosolization has also been stated to play a role in transmission (Gleeson *et al.*, 2007).

Coxiella burnetii is resistant to environmental conditions and remains in the environment after contamination for a long time. Q fever transmission to other species is particularly effective through contaminated air, which is the primary transmission method, due to its natural strong tolerance to harsh environmental conditions such as desiccation, heat, and antibacterial compounds. *Coxiella burnetii* has been isolated in ticks and found to play a role, ticks have been

a role as putative vectors and reservoirs for *C. burnetii* infection in animals (Fard and Khalili, 2011)*.*

2.6. Clinical signs of Chlamydia and Coxiella in human

2.6.1. Clinical signs of *Chlamydia abortus* in human.

Clinical cases of *C. abortus* infection in humans are considered to be rare. Exposure is not considered life-threatening in healthy people, although it has been observed in cases of pelvic inflammatory disease (Yin *et al.,* 2014). Pregnant mothers, on the other hand, are at risk of contracting *C. abortus*, which can be fatal to both the mother and the fetus. The disease can cause miscarriage, stillbirth, or premature delivery depending on when it is contracted. (Pospischil *et al.,* 2002)*.* Furthermore, unless the mother receives immediate medical attention, systemic complications may be fatal in pregnant mothers *(*Rodolakis *et al.,* 2010).

2.6.2. Clinical signs of *Coxiella burnetii* in human.

Medical symptoms of *C. burnetii* infection may be acute or chronic. The majority of Q fever cases, on the other hand, are asymptomatic and do not require hospitalization. A self-limiting febrile disease, atypical pneumonia, or hepatitis are the most common symptoms of symptomatic acute Q fever, with endocarditis being the most common symptom of chronic Q fever. Symptoms that are close to the flu may occur, but Q fever is not always diagnosed as such. In most cases, the illness manifests as either pneumonia or hepatitis. Chronic Q fever progresses as the infection becomes more severe. The persistent complications of the disease have been related to endocarditis, or inflammation of the aortic heart valves. People that are not suffering from recurrent Q fever have a higher chance of survival. (Anderson *et al.,* 2013)*.*

2.7. Clinical signs of *Chlamydia* and *Coxiella* in animals

2.7.1. Clinical signs of *Chlamydia abortus* in animals

Chlamydia abortus infection in animals are often asymptomatic and clinically unnoticeable. Young or pregnant animals, on the other hand, show clinical symptoms and even high mortality when exposed to stress factors. *Chlamydia abortus* cause chronic reproductive tract infection, placental insufficiency, and abortion in sheep, goats, and cattle (Lukac *et al.,* 2017). While ewes produce a vulval discharge during the last 48 hours of pregnancy, infected animals show no clinical symptoms prior to the abortion. Pathogenesis begins around day 90 of pregnancy, when chlamydial invasion of placentomes causes an inflammatory response, thrombotic vasculitis, and tissue necrosis, all of which occur during a period of rapid fetal development. Milder abnormalities can be seen in the fetal liver and lung, and there may be evidence of hypoxic brain injury in severe cases of placental destruction. In sheep, late-term abortion and the evacuation of necrotic fetal membranes are diagnostic signs. (Longbottom *et al.,* 2013).

2.7.2. Clinical signs of *Coxiella burnetii* in animals.

Despite the fact that Q fever in domestic ruminants is mostly asymptomatic, clinical manifestations such as abortions, stillbirths, infertility, and the birth of weak offspring have been recorded (Agerholm, 2013). Domestic ruminants are mostly subclinical carriers of *C. burnetii,* but they can shed bacteria in a variety of excretions including urine, milk, faeces, placental, and birth fluids. Other clinical signs include mastitis in ewes and does and inflammation of the uterus (Rahimi, 2011).

2.8. Diagnosis of *Chlamydia* and *Coxiella*

2.8.1. Diagnosis of *Chlamydia abortus*

The diagnosis of *C. abortus* can be made based on a history of an abortion in ewes and does, especially during the late pregnancy. The other approach to the diagnosis of *C. abortus* infection include clinical signs such signs of purulent to necroting placentitis characterized by vasculitis and abortions. Laboratory techniques including nucleic acid analysis by PCR, isolation of the bacterium by cell culture can be used for confirmatory diagnosis. Serological techniques such as IFT, Elisa, CFT and ICT have been used for detection of antibodies and antigens samples for conforming *C. abortus* infection in animals must be taken in appropriate transport medium for confirmation in the laboratory (Murcia-Belmonte *et al.,* 2019).

2.8.1.1 Bacterial culture:

For several years, proliferation of the infecting organism in cell culture and demonstration of characteristic *Chlamydial* inclusions was the best way of confirming the existence of chlamydial infection. However, in order to maintain the organism's viability prior to inoculation, this type requires sufficient transportation and cold-storage facilities. In addition, increasing and isolating species in cell culture requires time, and maintaining consistent high-quality laboratory methods is difficult. (Kaltenboeck *et al, 1997b*).

2.8.1.2 Serological tests:

Serological detection is often only useful for prevalence investigations. It can be difficult to diagnose a recent *Chlamydial* infection since most chlamydial infections do not induce significant antibody changes. The gold standard for identifying antibodies against *Chlamydiaceae* species in animals is the complement fixation approach, which uses crude or partially distilled preparations of *Chlamydiaceae*-specific lipopolysaccharide (Perez-Martinez *et al., 1986*).

2.8.1.3 Molecular tests:

The confirmatory diagnosis of *C. abortus* infection in sheep and goats can be achieved by using molecular techniques, these techniques include conventional PCR which detects the bacteria DNA after performing agarose gel electrophoresis. This PCR employs specific primers targeting 16S-23SrRNA. The methods use prop genes, which combines RFLP with the PCR to discriminate between the various species of *chlamydia*. Other molecular techniques such as real time –PCR and DNA micro array methods have also been used for detecting *C. abortus* DNA as marker for infection. Real time-PCR is usually preferred molecular method because of its high specificity, high throughout and ease of standardization (Chernesky M. A, 1999).

In some cases, the amplified PCR product can be sequenced further and analyzed by blast analysis to confirm *C. abortus* DNA. Further, phylogenetic characterization of *C. abortus* can be achieved by using the sequenced PCR-products. This is usually done to establish their genetic diversity (Huang *et al., 2*001).

2.8.2. Diagnosis of *Coxiella burnetii*

There are several methods for demonstrating *C. burnetii*, however the one to utilize is mostly determined by the type of material and the investigation's purpose. The diagnosis of *C. burnetii* can be made based on a history of an abortion in livestock, high fever and presence of tick. The other approach to the diagnosis of *C. burnetii* infection include laboratory techniques including nucleic acid analysis by PCR, isolation of the bacterium by cell culture can be used for confirmatory diagnosis. Serological techniques such as IFT, Elisa, CFT and ICT have been used for detection of antibodies and antigens samples for conforming *C. burnetii* infection in animals must be appropriately handled and transported to the intended laboratory for confirmation (Anderson *et al.*, 2013).

2.8.2.1 Bacterial culture:

Coxiella burnetii is mostly isolated by culture from blood, milk, liver biopsy, and fetal materials following abortion, albeit this method is used in just a few laboratories due to the significant risk of transmission to laboratory employees and the technique's limited sensitivity (Maurin *et al.,* 1999). Antibiotics complicate the isolation of *C. burnetii* in patients with persistent Q fever, thus a negative culture does not always indicate the absence of *C. burnetii* infection. *Coxiella burnetii* will not grow in standard bacteriologic media. Special culture methods must be carried out in a lab that meets biosafety level 3 requirements. Human embryo fibroblast cells, L cells, and green-monkey kidney cells have all been used to culture *C. burnetii.* (Anderson *et al.*, 2013).

2.8.2.2 Serological tests:

Serological tests such as the indirect immunofluorescence test, enzyme-linked immunosorbent assay, and complement fixation test are used to diagnose Q fever (Maurin *et al*., 1999). Serological diagnosis is simple and allows for the distinction of acute and chronic Q fever infections by evaluating both acute and convalescent sera. The presence of distinct IgG antibodies implies a recent *C. burnetii* infection or previous exposure, depending on antibody titers and phase variance. Enzyme-linked immunosorbent assays are preferred for practical reasons such as the ability to track a large number of sera and high sensitivity. (Panel *et al.,*2010).

2.8.2.3 Molecular tests:

The Polymerase Chain Reaction can be used to diagnose acute Q fever, particularly in the first two weeks after symptoms emerge but before antibiotics are taken. Polymerase Chain Reaction can be used on a variety of samples, including whole blood, serum, and tissues. The approach has a high sensitivity and provides quick results. Molecular approaches can be used to confirm the diagnosis of *C. burnetii* infection in sheep and goats, these techniques include conventional PCR which

detects the bacteria DNA after performing agarose gel electrophoresis. This PCR employs specific primers targeting IS1111transposase element of the organism. Other molecular techniques such as real time –PCR and DNA micro array methods have also been used for detecting *C. burnetii* DNA as marker for infection. In some cases, the amplified PCR product can be sequenced further and analyzed by blast analysis to confirm *C. burnetii* DNA (Maurin *et al.,* 1999).

2.9. Treatment of *Chlamydia* and *Coxiella* infections.

2.9.1. Treatment of *Chlamydia abortus* infection.

For many years, tetracycline has been the gold standard in anti-chlamydial therapy. Tetracycline is a low-cost, high-effective antibiotic. Macrolide antibiotics, particularly erythromycin and tylosin, have long been used to treat *Chlamydial* infections instead of tetracycline (Ridgway *et al*., 1997). The first of a new class of antibiotics known as ketolides, telithromycin, was recently evaluated to treat *Chlamydia. Chlamydia* pneumoniae and other respiratory species that causes community acquired pneumonia have been treated with telithromycin (Amin, M., *et al.,* 2019).

2.9.2. Treatment of *Coxiella burnetii* infection.

Even without treatment, the majority of acute Q fever cases resolve spontaneously. However, doxycycline, the most common medication for Q fever, should be provided to confirmed or suspected cases. Treatment should be given within the first 3 days of onset of symptoms since this shortens the illness and reduces the risk for developing severe complications. Doxycycline remains the first choice of treatment for acute Q fever, and doxycycline plus hydroxychloroquine (Anderson *et al.,* 2013).

2.10. Control of *Chlamydia* and *Coxiella* infections

2.10.1. Control of *Chlamydia abortus*

The basic principles and strategies for preventing and managing infectious diseases extend to *Chlamydiaceae* organisms. For boosting animal resistance and minimizing *Chlamydial* infection mortality, high-quality nutrition and husbandry, proper quarantine before combining animals of diverse origins, and stress reduction are all required. Antibiotics must be provided promptly and for a sufficient period of time if *Chlamydial* infection is confirmed. Vaccines against ruminant *Chlamydial* infections are being developed using whole *Chlamydial* antigen and subunit vaccines (Talaat, A. M., and Stemke-Hale, K. 2005). Heifers with clinically undetectable *C. abortus* infection have reduced fertility, and immunization with the recombinant *C. abortus* subunit vaccine boosted 6-week pregnancy rates after single artificial insemination to 83%, up from 50% in mock-vaccinated heifers (Talaat, A. M. and Stemke-Hale, K. 2005).

2.10.2. Control of *Coxiella burnetii*

Controlling the infection in the reservoir host, risk groups, and the environment should be the focus of prevention and control efforts. Measures for prevention and control of Q fever include creating public awareness about the sources of Q fever infection in domestic ruminant facilities, proper disposal of the placenta, afterbirths, fetal membranes, and aborted fetuses. It's best to stay away from raw milk and milk products. Animals from other countries should be isolated. Early detection of *C. burnetii* cases/antibodies by surveillance, as well as strict adherence to control measures (Anderson *et al.,* 2013). In Australia, a Q fever vaccine has been developed and has successfully protected persons who have been exposed at work. However, in many other nations, this vaccine is not commonly available. Animal vaccines have also been developed, but they are not generally available (Delsing *et al.,* 2019).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study area:

A cross-sectional study of 130 sheep and goat pastoralists was conducted in Kajiado County, Kenya. Residents of this county mainly depend on livestock rearing for livelihood especially for the rural population. Kajiado County is located in the southern region of Kenya and covers approximately 21,900 km^2 of land. It is divided into has five sub counties which include Kajiado north, central, east, west and south and these are further divided into 25 administrative wards.

From Kajaido east, the Kaputiei North and Kenyawa-Poka wards were chosen, while Ildamat and Matapato South were selected from Kajiado center. From Kajiado west, only the Iloodokilani ward was selected. The wards for this study were chosen based on livestock density and accessibility.
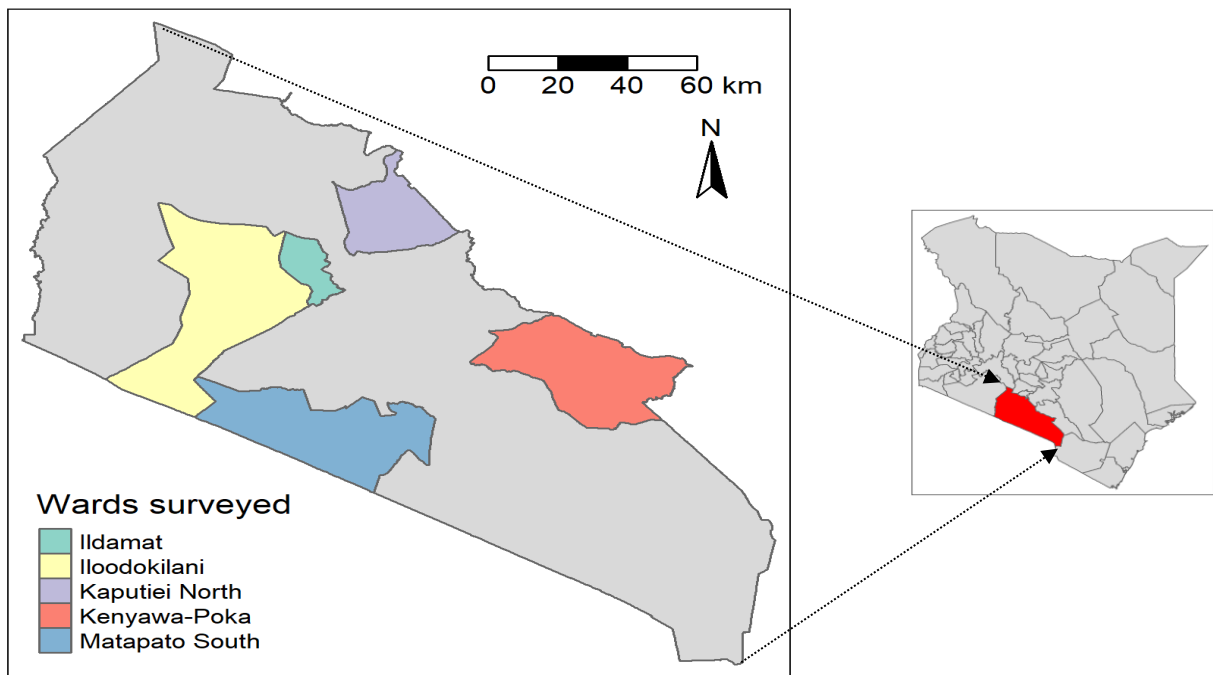
Figure 3: A map of Kajiado County, Kenya highlighting the various wards in the County.

3.2. Study design:

This study was a cross-sectional survey targeting 130 flocks of small ruminants kept by pastoralists in Kajiado county, Kenya during the wet season (June-august 2019). Whole blood samples were obtained from sheep and goats to test for *C. abortus* and *C. burnetii* using PCR. In addition, a retrospective assessment of pastoralists' flock management practices was conducted based on interviews.

3.3. Target population:

Pastoralists with medium and large flocks were the focus of the research. In this study, flocks containing more than 10 ewes and/or does, with a minimum overall flock size of 50 small ruminants. One of the survey's selection criteria was flock size. As a result, only for reproductive flocks were included in the study.

3.4. Sample size determination:

The sample size formula by Dohoo, et al; (2014) was used:

$$n = z_\alpha^2 * p * q / \mathcal{L}^2$$

Where:

n = the sample size required

p = a priori estimate of the proportion

q = 1-p

$Z\alpha^2$ = represents the desired level of statistical significance

L^2 = precision of estimate/allowable margin of error

At 95% confidence level, 10% precision and estimated prevalence of 50%, n= 96 flocks. Approximation to 100 and adjustment by 30% gives 130 flocks.

The number of sampled animals in a flock was determined by the formula for detecting disease from a finite population by *Dohoo, et al; (2014)*:

$$n = \left(1 - (\alpha)^{1/D}\right)\left(N - \frac{D-1}{2}\right)$$

Where;

n = the sample size required

α = 1-confidence interval (=0.05)

D = estimated minimum number of diseased animals in the population (population size x minimum expected prevalence of 20%).

N = population size (Minimum flock size will be 100)

Therefore, 12 sheep and goats (6 ewes and 6 does) per flock were sampled giving a total of 1,560 from 130 flocks.

3.5. Selection of the study participants:

One hundred and thirty small ruminant farmers were selected with the help of the staff from the Directorate of Veterinary Services in Kajiado County. Twenty-seven farmers were selected from Ildamat, and Iloodokilani, as well as 25 from Matapato south. The other 21 and 30 farmers were sampled from   Kenyawa-Poka and Kaputiei North respectively. Animals within the flock were selected through systematic random sampling. The sheep and goat populations were sampled separately.

3.6. Collection of blood samples:

Blood samples of sheep and goats were collected from the jugular vein after the animals had been properly restrained. A total of 4 mL of whole blood was collected in vacutainers and labeled with a waterproof marker pen. Thereafter, the samples were transported to the Department of Public Health, Pharmacology, and Toxicology of the University of Nairobi in a cool box with ice packs. The samples were stored in a freezer at -20°C.

3.7. Detection of *Chlamydia abortus* and *Coxiella burnetti* DNAs:

3.7.1. Extraction of DNA from the blood.

The whole blood samples of sheep and goats were obtained from the freezer and allowed to thaw at room temperature. Thereafter, genomic DNA of *C. abortus* and *C. burnetti* were extracted using Gene JET whole Blood genomic DNA purification Mini-Kit according to the manufacturer's guidelines (www.thermofisher.com). During the extraction of the genomic DNA from the whole blood samples, 20µl of proteinase K was pipetted into 1.5ml sterile Eppendorf tubes followed by the addition of 200µl of the whole into the same tube, and then mixed by vortexing briefly. Thereafter, 400µl of lysis solution was added to the proteinase K mixture, which was then thoroughly mixed by pulse-vortexing for 15 seconds to achieve a uniform suspension. To allow for digestion of proteins to occur, the sample was incubated at 56^0C for 10 minutes in a water bath. After the digestion, 200 µL of absolute (96-100%) ethanol was added to the homogenous lysed blood samples and the mixture was vortexed for 15 seconds. Then, the homogenous mixture was transferred to the spin column. To bind the DNA, the column cap was closed and the lysed blood sample was centrifuged at 10,000 rpm for 1 minute. The collection tube containing the flow-through solution was discarded and the spin column was placed into a new 2ml collection tube. After the binding of DNA, 500 µL of wash buffer WB I (with ethanol) were added into the column

and centrifuged at 10,000rpm for 1 minute. The flow-through was discarded and the column was placed back into the collection tube. Once again, 500 µl of wash buffer WB II (with ethanol) was added into the DNA Mini kit column, the column cap closed and then centrifugation was done at 15,000rpm for 3 minutes. The purification column was re-spined for 1 minute at a maximum speed of 14,000rpm, following the protocol of the manufacture. The column was placed to a sterile 1.5ml micro-centrifuge tube after the flow-through solution was emptied from the collection tank.

To elute the DNA in the Mini kit column, 100 µl of elution buffer were added in the center of the column membrane and the mixture was incubated for 2 minutes at room temperature. Thereafter, the mixture was centrifuged for 1 min at 15,000rpm and the eluted DNA was recovered in 1.5 ml sterile Eppendorf tubes. The eluted DNA samples were labeled with a waterproof marker- pen and then stored at -20°C pending analysis by PCR.

3.7.2 Amplification of *Coxiella burnetti* DNA by PCR.

To identify the *C. burnetti* DNA extracted from whole blood, the specific nucleotide primers CB1a (100 µM) forward F-trans 5'-TATGTATCCACCGTAGCCAGTC-3' and reverse CB2a (100 µM) R-trans 5'-CCCAACAACACCTCCTTATTC-3' *(Macrogen, Netherland)* was used to amplify IS1111transposase element of the organism. The Veriti 96 well thermos-cycler was used for amplification of the target DNA. The PCR amplifications were performed in 20 µl reactants containing 10 µl master mix, 0.24 µl forward primers (100 M), 0.24 µl reverse primers (100 M), 5.52 µl DNase/RNase-free distilled water, and 4 µl template DNA. Initial DNA denaturation was performed at 95°C for 5 minutes, followed by 40 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 60°C, and extension for 1 minute at 72°C. For the final extension, the reaction mixture was kept at 72°C for 1 minute. The PCR amplicons were electrophoresed in a 2%

agarose gel stained with ethidium bromide (0.5 µg/ml) and visualized with a computerized UVP GelMax Imager.

3.7.3 Amplification of *Chlamydia abortus* DNA by PCR.

To identify the *Chlamydia abortus* DNA extracted from whole blood, the specific nucleotide primers CA1b (100 µM) forward F-IS-rRNA 5'-AACCTGCAAAGGAGAGAGGC-3' and reverse CA2b (100 µM) R-IS-rRNA 5'-CCAGCTCGCCGAAGATTACT-3' reverse *(Macrogen, Netherland)* used to amplify 16S-rRNA-16S-23S rRNA-is (KX870500.1) of the organism. The Veriti 96 well thermos-cycler was used for amplification of the genes. The PCR amplifications were performed in 20 µl reactants containing 10 µl master mix, 0.24 µl forward primers (100 M), 0.24 µl reverse primers (100 M), 5.52 µl DNase/RNase-free distilled water, and 4 µl template DNA. Initial DNA denaturation was performed at 95°C for 5 minutes, followed by 40 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 56°C, and extension for 1 minute at 72°C. For the final extension, the reaction mixture was heated to 72°C for 1 minute. The PCR amplicons were separated on a 2% agarose gel stained with ethidium bromide (0.5 µg/ml) and visualized in a computerized UVP GelMax Imager.

3.8. Questionnaire survey:

Structured questionnaire was developed and administered to assess the practices of pastoralists to find out the risk factors which could contribute to or limit the spread of *C. abortus* and *C. burnetii*, among animals. The questionnaire contained questions covering flock dynamics, risk factors associated with *C. abortus* and *C. burnetii*, in flocks with specific questions covering flock size, flock composition, history of occurrence of abortions in the flocks, sharing of common grazing fields and watering points and the introduction of new animals into the flock.130 pastoralists were interviewed orally and their responses entered into the ODK. The epidemiological data collected in the questionnaires included knowledge attitude and practices of the farmers. In addition, data on awareness of farmers to zoonotic disease were obtained.

3.9. Data management and analysis.

3.9.1. Data management:

The questionnaire was collected and saved in the Open Data Kit (ODK) (https://opendatakit.org/) software format. The raw data from molecular investigation and ODK questionnaire were entered into a Microsoft excel (Microsoft Excel®). The data was cleaned and coded in a computer spreadsheet, then exported to (IMB SPSS) v.20 for analysis.

3.9.2. Data analysis:

Proportions were determined for categorical variables and presented as a percentage of the overall number. The frequency tables in SPSS software were used to do descriptive statistics. Thereafter, logistic regression analysis was used to find out possible risk factors that were associated with *C. abortus* and *C. burnetii*. Odds ratios were used to show the degree of association between the identified risk factors and *C. abortus* and *C. burnetii* infections.

3.10. Ethical approvals and considerations.

The farmers of the sampled households in Kajiado County were informed about the study, and thereafter, gave their approval for the sampling of sheep and goats and consent was obtained from them. This study was done according to ethical guidelines for the use of animal samples permitted by International Livestock Research Institute in Nairobi, which is recognized by the National Commission for Science, Technology, and Innovation (NACOSTI), the Kenyan government, under the reference of ILRI-IREC2019-18.

CHAPTER FOUR: RESULTS

4.1. *Coxiella burnetii* DNA detected in blood.

A total of 1022 blood samples collected from sheep and goats in the different wards in the Kajiado county were analyzed for C. *burnetii* using PCR. *Coxiella burnetii* DNA was not detected in all the blood samples analyzed by PCR. The failure to detect *C. burnetii* DNA was observed as absence of a PCR band corresponding to 687 bp in the gel image (figure 4).
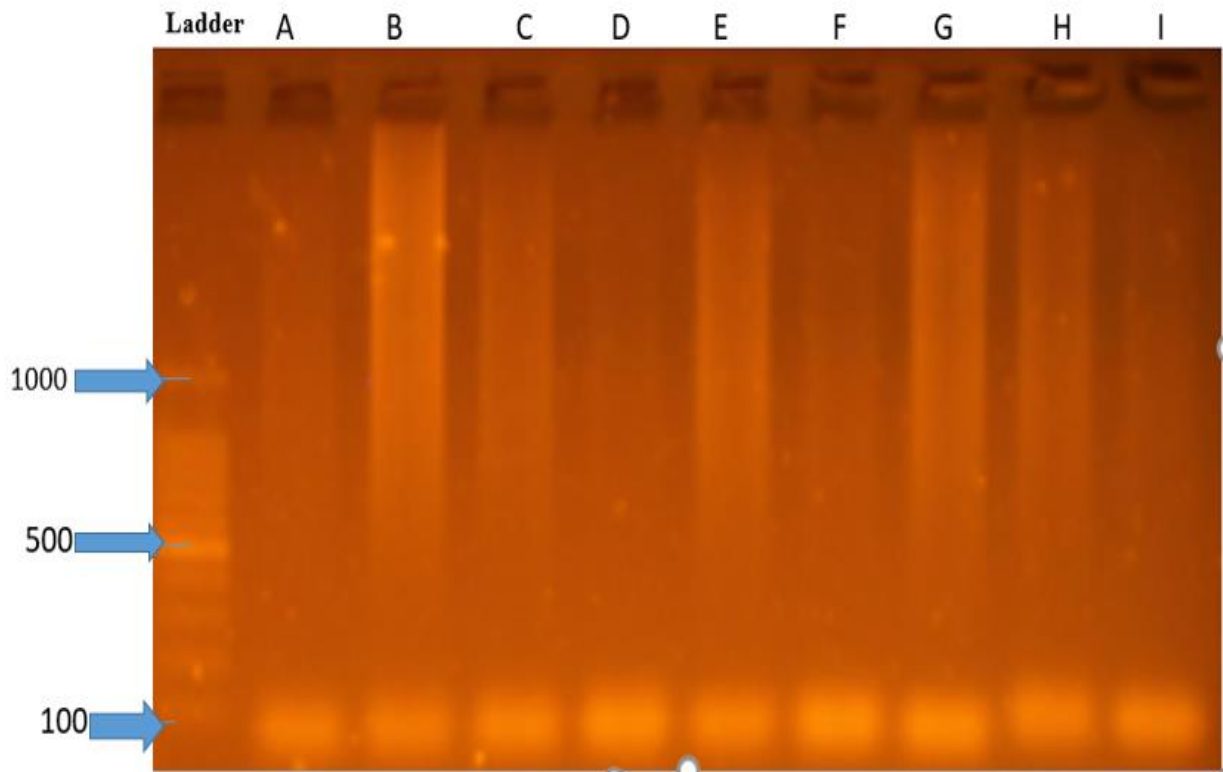
Figure 4: Blank Gel-image of representative samples analyzed by conventional PCR. The absence of band indicated failure to detect *C. burnetii* DNA.

4.2. *Chlamydia abortus* DNA detected in blood.

A total of 347 blood samples uses analyzed by PCR in order to detect *C. abortus* DNA. In particular, 148 samples from sheep were analyzed while 199 sample were analyzed from goats. In over all, 86 (24.8%) of the analyzed samples were positive for *C. abortus* on analysis by PCR. The positive samples were observed as PCR band corresponding to 415 bp in the gel image (figure 5).
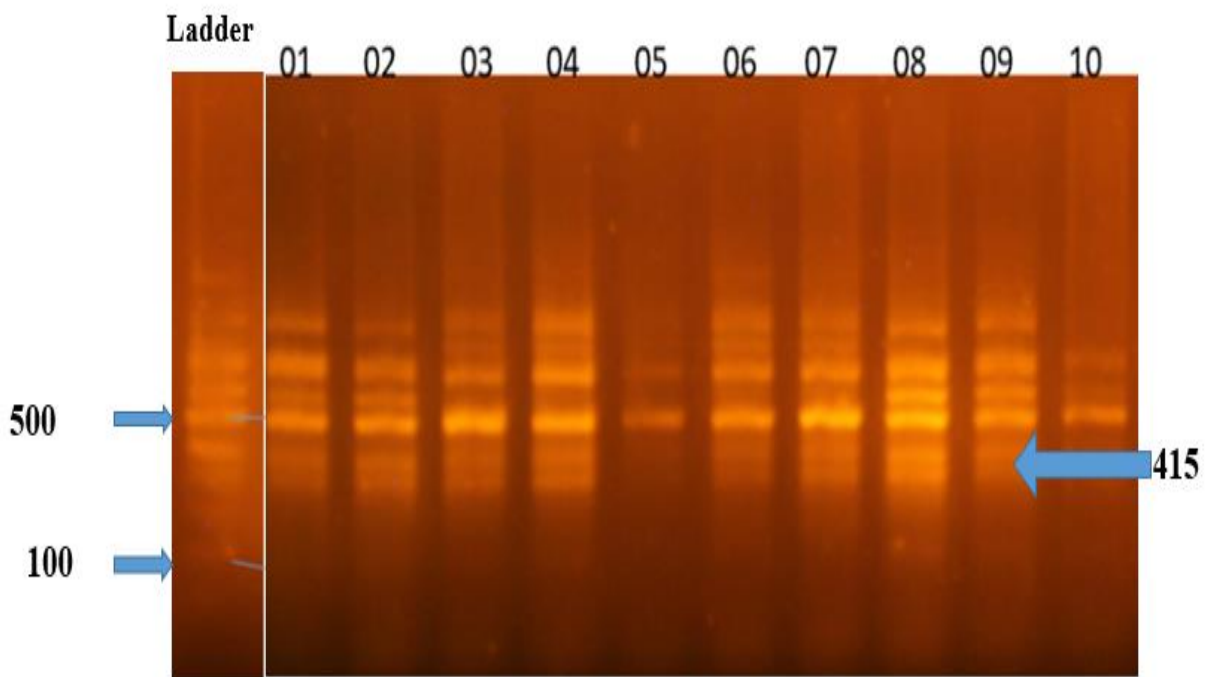
Figure 5: Gel-image of representative samples analyzed by conventional PCR for *C. abortus* 1-10 samples are representative of the samples analyzed.

Over all, *C. abortus* DNA was detected in 86(24.8%) samples analyzed in all the 5 wards. A higher proportion of *C. abortus* DNA was detected in goats (28.1%) than in sheep (20.3%) as shown in table 2. However, as shown in table 1 the difference in proportions of *C. abortus* DNA detected in the two species was not statistically significant (P <0.0.5).

Table 1: Number of sheep and goats blood samples tested for *C. abortus* and *C. burnetii* by PCR.

Target pathogens	Sheep	Goats	Positive cases	P-value	Confidence Interval(95%)
Coxiella burnetii	577	445	0	-	-
Chlamydia abortus	148(30)	199(56)	86	0.0865	0.161-0.216

Key: The numbers in parenthesis are those samples that tested positive sheep and goats respectively.

With respect to detection of *C. abortus* DNA in sheep and goats reared in the five wards, the highest detection rates were observed in Ildamat (34.9%) and Iloodokilani (31.6%). The lowest detection rate was observed in Mathapato South (15.4%). The detection rates for all other wards are shown in table 2.

Table 2: *Chlamydia abortus* DNA detected in blood samples of the selected wards

Ward	Sample size	Positive cases(%)
Ildamat	43	15 (34.9)
Mathapato_south	46	5 (10.9)
Iloodokilani	155	49 (31.6)
Kenyawa_poka	65	10 (15.4)
Kaputiei_north	38	7 (18.4)
Total samples	347	86 (24.8)

Key: The numbers in brackets are the proportion of those samples that tested positive.

4.3 *Chlamydia abortus* **DNA detected in sheep and goats in the selected wards.**

As shown in table 3, a higher proportion of the samples with *C. abortus* DNA were observed in goats (42.9%) than in sheep (18.3%) for three wards namely, Ildamat, Iloodokilani and Kenyawa poka. There was statistical significant difference in the proportion of positive samples from goats and sheep reared in Iloodokilani (P <0.0.5). Otherwise, there was no significant difference observed in the other wards (P >0.0.5).

Table 3: Proportion of sheep and goats testing positive for *C. abortus* DNA per ward

Wards	Sheep (%)	Goats (%)	P-value
Ildamat	5 (25)	10 (43.5)	0.1920
Mathapato south	3 (13)	2 (8.7)	0.6349
Iloodokilani	13 (18.3)	36 (42.9)	0.0005 *
Kenyawa poka	4 (25)	6 (12.2)	0.2795
Kaputiei north	5 (27.8)	2 (10)	0.1552

Key: The numbers in brackets are the proportion of those samples that tested positive. * Indicate statistically significant value.

4.4. Small ruminant farmers' demographic characteristics.

The farm demographics are shown in table 1 below. Out of the sample of the130 respondents, 87.7% were male while 12.3%were females. As shown in table1 below, out of 130 respondents in the five wards, only 4.6 % were less than 30 years old, while 46.9 % were between 30 to 50 years old. The study also found (48.5%) of the respondents were over 51 years indicating that older people were more engaged in the household activities such as small ruminant rearing. Only 0.8% of respondents were single, while 95.4% were married, and the other 3.8 % were divorced. The farmers with formal education, (30.8 %) were found to have completed university education. Only 13% of those sampled in the five wards had no formal education. The average household size of the 130 respondents was 6 individuals with a minimum of 1 person in households. The maximum household size was 20 individuals. With respect to the sheep flock, 54.6% of the flocks reared in

Kajiado were Cross-breed while 33.3% and 11.5% were Doper and Red Maasai sheep respectively. Only 0.6% was Somali-breed. On the other hand, 50.3% of the flocks of goats were Cross-breed, while 40% were Galla goats. The other 9% and 0.7% were Small East African and Alpine goats respectively. Majority of the respondents (90.2 %) practiced mixing farming whereby sheep and goats were reared together with cattle. The other 0.4 % of the respondents cultivated crops in addition to rearing cattle, sheep and goats. The study also established that 2.3 % and 6.8 % of the respondents were involved in livestock trade and other business activities respectively.

Table 4: Description of demographic information of the 130 respondents in Kajiado county.

Variables factors	Categories	Frequency	Percent(%)
Sex of household head	Male	114	87.7
	Female	16	12.3
Age of household head	less than 30	6	4.6
	30 to 50	61	46.9
	over 51	63	48.5
Marital Status	Single	1	0.8
	Married	124	95.4
	Widow	5	3.8
Education of household head	Non formal education	17	13
	Primary school	43	33.1
	Secondary school	30	23.1
	College/university	40	30.8
Farming activities	Small-ruminant rearing	129	48.5
	Cattle rearing	111	41.7
	crop farming	1	0.4
	trade live activities	6	2.3
	trade crop products	1	0.4
	other business	18	6.8
sheep breed	Red maasai	20	11.5
	Doper	58	33.3
	Somali	1	0.6
	Cross breeds	95	54.6
goat breed	Galla	58	40
	Small east African	13	9
	Alpine	1	0.7
	Cross breeds	73	50.3

4.5. Farm management practices of pastoralists:

The management practices of 130 respondents in Kajiado County are given in table 2 below. A study of the flock sizes revealed that 61.5% of the respondents had flock sizes less than 100. The other 26.9 % of the farmers interviewed had flock sizes of between 100 and 200. The proportion of respondents with large flock sizes of the respondents having over (> 200) was 11.5%. During the interview, it was established that 96.5 % of the respondents practiced extensive farming systems where livestock are grazed communally in an expensive pasture. The other 3.1% practiced semi-intensive system. In this case, livestock were reared both by grazing them on open pastures and sometimes in fenced areas. Intensive farming system was not practiced in the study area. All the respondents interviewed were found to rear their sheep and goats in communal livestock keeping system.

Approximately 42 % of the respondents used communal watering pan while the other 40% used rivers as watering points for their livestock. The other watering points for the pastoralists included wells (13.8%) and o piped water system (3.8%). the study also found that 83.8% of the respondents used wells for watering their livestock during dry season. The other 13.8% used water pan as a source of drinking water for the livestock. Fewer respondents interviewed used rivers (1.5%) and piped watering system (0.8%) as watering points for livestock. With regards to breeding system, 81.5% of the respondents used their own rams and bucks for breeding. The remaining 17.7% of the respondents bought male animals for breeding from other sources. Only minority group (0.8%) borrowed breeding males from their fellow farmers.

This study also established that 70% of the respondents don't move with all of their livestock during the dry season while in search of pasture. In particular, they only migrate with cattle leaving sheep and goats behind. For flock management, 3.1% of respondents were found to replace their flocks after selling some. Majority (96.9%) did not replace their flocks after selling some of their animals. The trading of sheep and goats in livestock markets indicated that pastoralists use sheep and goats for their livelihoods.

Table 5: Results of farm management practices of pastoralists in Kajiado County Kenya.

Variables factors at farm level	Categories	Frequency	Percent (%)
Flock size	< 100	80	61.5
	100-200	35	26.9
	>200	15	11.5
Grazing system	Semi-intensive	4	3.1
	Extensive	126	96.9
Source of water-raining season	Well	18	13.8
	River	52	40.0
	water pan	55	42.3
	piped water	5	3.8
Source of water - dry season	Well	109	83.8
	River	2	1.5
	water pan	18	13.8
	piped water	1	.9
Breeding system	Raised my own	106	81.5
	Bought	23	17.7
	Borrowed	1	.8
Animal movement	No	91	70
	Yes	39	30
Animal replacement	Yes	4	3.1
	No	126	96.9

4.6 Pastoralists reported reproduction challenges.

To assess the risk factors responsible for reproductive challenges in the farms, respondents were interviewed by administering questionnaires. Abortions, still births cases and various infections and droughts were the reproductive challenges reported by the farmers. In particular, 56.2% of the farmers interviewed reported abortions in the flocks as one of the reproductive challenge. Some of the respondents reported still birth (74.6%) to be a reproductive challenge in their flocks. On the other hand, 9.8% of pastoralists interviewed believed that these reproductive challenges are associated with drought. The other 90.2% of the pastoralists believed that these abortions and still birth were as results of infections such as brucellosis, Rift valley fever and tick borne diseases.

Table 6: Pastoralists reported reproductive challenges in their flocks (sheep and goats) in Kajiado county Kenya (n=130)

Description of items	Number reporting "yes"	Proportion (%)
Farmers experiencing abortion	73	56.2
Farmers experienced stillbirths	97	74.6
Causes of abortion	13	9.8
Drought	74	55.6
Infectious	17	12.8
Rift valley fever	16	12
Brucella	13	9.8
Tick borne diseases		

4.7. Contribution of Farmers' practices to Public health risk.

Practices related to animal slaughter, consumption of raw milk as well as meat and blood was also assessed in these farms. The study found that majority of the pastoralists (71.5) slaughter sheep and goats at home, indicating that there is risk of exposure to dangerous infections such as *C. abortus*. Only 7.7% of the respondents consumed raw milk. The rest consumed boiled milk. It was also that 70.8% of the farmers boil their milk before fermentation. The other 29.2% fermented their milk without boiling. Majority of the respondents (79.2%) do not consume raw meat, with only 20.8% reported to have consumed raw meat occasionally. Slightly more than a half (52%) of the pastoralists was reported to have consumed raw blood from sheep and goats on occasionally. Minority (3.8%) of the respondents used protective gear while handling aborted animals with only 5.4% reporting to have used protective gear intermittently.

Table 7: Contribution of practices of farmers to public health risks

Variables	Categories	Category Frequency	Category Percent (%)
Slaughter sheep/goat at home	Always	32	24.6
	Occasionally	61	46.9
	Never	37	28.5
Drinking raw sheep/goat milk	Always	2	1.5
	Occasionally	8	6.2
	Never	120	92.3
Fermented milk from raw milk	Always	18	13.8
	Occasionally	20	15.4
	Never	92	70.8
Eat raw sheep/goats meat	Always	1	0.8
	Occasionally	26	20
	Never	103	79.2
Taking raw blood	Always	2	1.5
	Occasionally	66	50.8
	Never	62	47.7
Protective gear(abortion)	Always	5	3.8
	Occasionally	7	5.4
	Never	118	90.8

4.8 Awareness of farmers on risk of zoonotic diseases.

During questionnaire administration, famers were asked whether they are aware of zoonotic diseases. Overall, 27.7% of the respondents were aware that same diseases are zoonotic and could be transmitted from animals to human. The other remaining respondents had no idea of the zoonotic diseases. There were respondents (30%) who were aware that some diseases can be transmitted to human by consumption of raw milk. The rest were not aware of this risk. Generally, pastoralists interviewed were also aware of the occurrence of brucellosis, Rift valley fever, anthrax and rabies (table 8). Nevertheless, majority of those interviewed were not aware of occurrence of *C. abortus* infection in human.

Table 8: Analysis of awareness with regards to zoonotic diseases among the farmers (n=130)

Description of items	Number reporting "yes"	Percentage (%)
knowledge of zoonotic diseases among farmers	36	27.7
Knowledge of zoonotic diseases through animal product among farmer	39	30
Brucella	14	10.8
Anthrax	13	10
Rift valley fever	5	3.8
Rabies	3	2.5
Q fever	1	0.8

4.9 Univariate risk analysis Result:

On univariate risk analyses, Water point during raining season (OR=1.58; P=0.05), and reports of previous abortion cases in flocks (OR= 0.83; P=0.02) were found to be statistically significant risk factors. Other risk factors, that were statistically significant were farmers have previous knowledge of diseases (OR= 0.81; P=0.01), and consumption of fermented raw milk by household (OR=1.22; P=0.53).

4.10 Multivariate risk analysis result:

As shown in table 10, watering point during rainy season, farmers reporting previous experiences of abortion in flocks and consumption of raw fermented milk were found to be statistically significant risk factors for the occurrence of *C. abortus* infection (P<0.05). Sheep and goats drinking water from rivers were 1.32 times more likely to acquire for *C. abortus* infection than those drinking water from piped water (OR=1.32, P=0.02). Sheep and goats drinking on water-pans as source of drinking water were 1.57 times more likely to be infected with *C. abortus* than those drinking water from piped water (OR=1.57, P=0.02). Multivariate analysis also established that pastoralists consuming raw fermented milk were 1.25 times more likely to be infected with *C. abortus* than those who do not consume fermented raw milk (OR 1.25, P=0.05). Finally, the flocks that never aborted are 0.84 times less likely to be infected with C. *abortus* infection (OR0.84, P=0.02).

Table 9: Univariate analysis of _Chlamydia abortus_ outcome variable among 130 sheep and goats' farms in Kajaido, county Kenya.

parameters	Category	Estimate	Std. error	Chi-square	P-Value	Odd Ratio
Water points during raining season	Well	0.28	0.25	1.26	0.26	1.32
	River	0.21	0.23	0.83	0.36	1.24
	Water pan	0.46	0.23	3.83	0.05	1.58
	Piped water	Reference	-	-	-	1
Abortion experience	No	-0.19	0.08	5.66	0.02	0.83
	Yes	Reference	-	-	-	-
Infectious disease knowledge	No	-0.22	0.08	7.10	0.01	0.81
	Yes	Reference	-	-	-	1
Fermented raw milk	Always	0.19	0.12	2.76	0.053	1.22
	Occasionally	-0.11	0.11	0.86	0.35	0.90
	Never	Reference	-	-	-	-

Table 10: Multivariate logistic model for variable associated with *Chlamydia abortus* molecular positivity for 130 sheep and goat farms, Kajaido county Kenya.

Parameters	Category	Estimate	Std. Error	Chi-square	P-Value	Odd Ratio
Water source during raining season	Well	0.34	0.21	2.57	0.11	1.41
	River	0.28	0.20	1.9	0.16	1.32
	Water pan	0.45	0.20	5.27	0.02	1.57
	Piped water	Reference	-	-	-	1
Experience of abortion	No	-0.170	0.0753	5.114	0.02	0.84
	Yes	Reference	-	-	-	1
Fermented raw milk	Always	0.23	0.11	3.89	0.05	1.25
	Occasionally	-0.051	0.11	0.24	0.63	0.95
	Never	Reference	-	-	-	1

CHAPTER FIVE: DISCUSSION

This study is aimed at identifying the *C. abortus* and *C. burnetii* pathogens infecting sheep and goats in selected areas of Kajaido County, Kenya. *Coxiella burnetii*-DNA was not detected in blood samples of both sheep and goats. Nevertheless, a previous serological test performed in Kajiado County revealed the presence of *C. burnetii* antibodies with a prevalence of 57.5% and 83.1% sheep and goats respectively (Njeru *et al.*, 2016). Therefore, since the bacterium has not been detected in small ruminants in Kajiado County, the majority of seropositive cases detected previously were most likely to be due to a previous exposure to *C. burnetii.*

Based on the type of sample and the goal of the examination, serologic and molecular method has been used for the diagnosis of Q fever (Muskens *et al.,* 2012*).* Serodiagnostic methods for detecting Q fever infection in animals and humans include indirect immunofluorescence, complement fixation examination, and ELISA. For the detection of animals shedding the bacterium, both conventional PCR and real time PCR have been used as a quick and responsive tests. The main sources of infection are the uterus and mammary glands (Muskens *et al.,* 2012*).* Detection of antibodies to *C. burnetii* in animals suggests a previous exposure, while the presence of pathogen genetic material indicates acute infection. Furthermore, there has been no correlation found between antibody response and bacterial excretion (Gangoliya *et al.*, 2019). A study done in 2014 in Korea reported Q-fever infectivity rate using PCR to be lower than that of ELISA seropositivity rate. A possible reason for this discrepancy is that ELISA can detect previous *C. burnetii* infections in individual goats that lack the bacterial DNA but still have circulating antibodies. Furthermore, it has been reported that PCR can only detect acute early infections when the bacterial DNA is the only one present but not circulating antibodies (Jung *et al.,* 2014*).*

The findings of this research confirmed the existence of *C. abortus* infection through molecular technique among the sheep and goats in Kajiado, Kenya. This is the first molecular analysis-based study of *C. abortus* infection in sheep and goats in Kenya. *Chlamydia abortus* is found in small ruminants all over the world, according to several reports. For the current literature review of other studies in Africa, the prevalence of *C. abortus* infection in small ruminant ranges between 3.3 % and 35.4%. This study revealed a prevalence of 24.8%, which is within the range of previous studies although most of those studies were based on serological tests while this study used molecular technique. Similar investigations in other settings in other African nations, on the other hand, discovered a wide range of prevalence for *C. abortus* infection in small ruminants. Seroprevalence studies of *C. abortus*-infection in sheep and goats in Ethiopia revealed a prevalence of 9.88% (Tesfaye *et al.,* 2020*).* The prevalence of *C. abortus* has been reported to be 35.4% in Algeria (Merdja *et al.,* 2015), 13.7% in Egypt, (Selim *et al.,*2021*)*, and 12.9% in Tunisia (Barkallah *et al.,* 2018*).* In Namibia, a seroprevalence of 28.2% has been reported for C. *abortus*-infection (Samkange, *et al.,* 2010*).* Additionally, another survey conducted in Zimbabwe revealed a prevalence of 22% for *C. abortus*-infection in small ruminants (Bhandi *et al.,* 2019*).* Other serological survey conducted in central Africa particularly, Equatorial Guinea reported that the prevalence of *C. abortus* is 19.9% (Loureiro *et al.,* 2017).

The prevalence of *C. abortus* in sheep and goats documented for this study was 20.3% and 28.1% respectively consistent with prevalence reported in Zimbabwe. Nevertheless, lower prevalence of 10% and 2% for the infection in sheep and goats respectively has been reported in Iraq (Majid*, et al.,* 2018). According to a recent report in India, the prevalence of *C. abortus* infection in sheep has been reported to be 10.60% and 22.46% in goats *(*Chahota *et al.,* 2015). In Belgium, a serological examination of the sheep and goats revealed a prevalence rate of 0.68% and 52.9%

respectively (Yin *et al.,* 2014). Although this study reported a higher prevalence of *C. abortus* in goats (28.1%) than in sheep flocks (20.3%); this difference was not statistically significant at (P<0.0.5) indicating that *C. abortus* infection had no association with the animal species. The finding was in agreement with a recent study carried out in Iran, which reported a prevalence of 26.7% in sheep and 24% in goats (Esmaeili *et al.,*2015). Since this difference was not statistically significant, it is possible that C. *abortus* infection had no relationship with animal species as observed in the current study. Nevertheless one study in Ethiopia reported a higher seroprevalence of *Chlamydiosis* in goats than sheep (Tesfaye *et al*., 2020). This difference may be explained by the differences in geographical location, disease control methods and flock size. The prevalence of the disease varied slightly among the animals sampled from five different wards. Ildamat has the greatest prevalence rate (34.9%) with Iloodokilani having the second highest prevalence while Matapato South has the lowest. The difference was statistically significant (P= 0.013) and this difference could have been due to the different farming practices by the farmers in these words. Otherwise, further study may be necessary to explain the actual farming practices that may have contributed to this difference in prevalence of *C. abortus* infections in theses wards.

The risk factors associated with the occurrence of *C. abortus* from the univariate analysis included watering-point during the rainy season, farmers reporting experiences of abortion in their flocks, farmers having previous disease knowledge, and farmers' consumption of fermented raw milk. Multivariable analysis of the risk factors associated with the occurrence of *C. abortus* were water source during raining season, farmers reporting experiences of abortion in their flocks and household consumption of fermented raw milk. There was no significant association for farmers with prior disease knowledge. It is expected that confounding effect of flock size could have contributed to a lack statistical difference in the association. A study by (Merdja, *et al.,* 2015)

considered the intensive management system, and high prevalence of chlamydial infection as significant risk factors because these factors increase the probability of infection resulting from risk of contacts between animals and the surrounding contaminated with the pathogen.

A significantly higher positivity of *C. abortus* was found in animals watered from the rivers and water pans during rainy season. When compared to sheep and goats watered by piped water, small ruminants drinking from rivers were 1.32 times more likely to test positive for *C. abortus* (OR=1.32, P=.02). Small ruminants watered from water pans were 1.57 times more likely than sheep and goats watered from piped water to test positive for *C. abortus* (OR=1.57, P=.02). The reason for this observation could be that many animals congregate in rivers and watering pans to drink, increasing contact between sick animals and so raising the risk of disease transmission and introducing bacteria into the ecosystem. *Chlamydia* bacteria are shed in nasal secretions, vomit, semen, vaginal discharge, and saliva and are considered to be resilient in the environment. The infection can be spread via the respiratory, ocular, or oral routes in animals. At room temperature, *Chlamydia* EB can survive for 10 to 15 days in feces. They can also survive for months in dried animal droppings (Talafha and Ababneh., 2012).

Farmers reporting experiences of abortion in their flocks was a significant risk factor for this study. Sheep and goat flocks that have not experienced abortion are 0.84 times less likely to be positive for *C. abortus* than flocks that had abortions (OR.84, p=.02). *Chlamydia abortus* plays a significant role in abortions of sheep and goat in Kenya indicating that any cases of abortions observed in these flocks can be explained by presence of the infection in the affected flocks. In fact, (Longbottom *et al.,*2013)*, (*Ababneh *et al.*, 2014) and (Barati *et al*., 2017) have reported *C. abortus* as one of the infectious agents of abortions in sheep and goats. This reported supported the findings of this study in which 56.2% of the farmers interviewed reported to have experienced

abortions in their sheep and goats' flocks and 90.2 % of these farmer believed that the abortions in flocks is caused by infections.

This study also found that household intake of fermented raw milk was a significant risk factor. Raw fermented milk was 1.25 times to be more likely contaminated with *C. abortus* compared to those who never fermented their raw milk from sheep and goats (OR 1.25, p=.05). Other respondents (29.2%) were found not to boil milk before they ferment it for home consumption. Consequently, the likelihood of exposure to the bacteria when the community consumes un-boiled fermented milk could be high with the risk of acquiring the infection when un-boiled milk is consumed. (Rodolakis *et al.,* 2010), and (Barkallah *et al.*, 2018) have reported that consumption of raw un-boiled fermented milk could increase the risk of human infection with this pathogen since *C. abortus* is a zoonotic disease. Risk factors such as flock size, grazing system, watering-point during dry season, breeding system, animal movement and flock replacement were not significantly associated with the detection of *C. abortus*.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusion:

Based on the findings of this report, the following conclusions were made;

1. Sheep and goats reared in Kajiado County are not clinically infected with *C. burnetii* but previous exposure to the infection in these flocks cannot be ruled-out.

2. Sheep and goats reared in Kajiado County are infected with *C. abortus*

3. Use of communal watering-points during the rainy season, reports of previous abortions in the flocks by farmers, and household consumption of fermented raw milk are possible risk factors for occurrence of *C. abortus*-infection.

6.2. Recommendation:

1. Community awareness on the infections and the risk factors responsible for their occurrence in the flocks in the County is recommended.

2. The government must put in place disease control measures, such as *C. abortus* immunization, which is currently not being done in the county.

3. Because *C. abortus* is one of the most common causes of abortion, more research is needed to determine the influence of this disease on animal production and losses.

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APPENDIX 1: Questionnaire and observational assessment for risk factors:

Date of interview……………………………………………. Flock ID……………………………

Name of interviewer: ………….....………………………………………………………….……

Sub-county: ……………………………… ……………Ward: …. …..………..…………………..

Respondents' personal information:
Name of respondent (optional) ……………………………..……………………………........

Age (years): ……………………………. No. of dependents': ………………………………...

Sex (Tick appropriate box): ☐ Male ☐ Female

Civil status: ☐ Single ☐ Married ☐ Widow/widower ☐ Divorced

Level of education:

None ☐

No formal but literate ☐

Primary school ☐

Secondary school ☐

College/university ☐

Scio-economic status of Respondents:

What are the household activities? (Rank-answer question)

Small-ruminant rearing	
Cattle rearing	
Crop farming	
Trading livestock products	
Trading crop products	
Other business (neither related with livestock nor with crops)	

What is the main reason for keeping sheep and goats?

	SHEEP	GOATS
Regular cash income		
Insurance against emergencies		
Meat		
Celebrations		
Drought tolerance		
Diseases resistance		
Other, please specify		

64

Flock model questions:

1. Number of livestock kept:

  (i) Total number of sheep: …...................…….…… (Number will be 0 if don't have)

  (ii) Total number of goats…………………………... (Number will be 0 if don't have)

2. Breeds of sheep:

  ☐ Red maasai

  ☐ Dorper

  ☐ Somali

  ☐ Other breeds (Specify)…………………………………

3. Breed of goats:

  ☐ Galla

  ☐ Small east African

  ☐ Alpine

  ☐ Other breeds (Specify)…………………………………

Farm management system

1. Type of management for sheep and goats:

  ☐ Intensive

  ☐ Semi-intensive

  ☐ Extensive

2. Source of water

	Rainy season	Dry season
Own well		
Other pastoralist well		
Borehole/well		
River		
Roof harvested rainfall		
Water pan		
Piped water		
Other, please specify (free text)		

3. How many animals entered in the flock(s) during the last twelve months?

4. Regarding your breeding male how did you get it?

Raised my own	
Bought	
Rented	
Borrowed	

5. animal movement for water and pastures due to droughts.

4. a) Have you experienced abortion in your flocks in the last 12 months?   Yes   No

b) If yes, number of abortions in:

(i) Sheep…………………...

(ii) Goats…………………...

5. How often do you wear gloves when handling aborted material?

☐ Always

☐ Occasionally

☐ Never

5. Please indicate causes of abortion in sheep and goats:

☐ unknown

☐ Drought

☐ Fly bites

☐ Infectious disease without specifying any of them

☐ Toxoplasmosis

☐ Tick borne diseases

☐ Rift valley fever

☐ PPR

☐ Brucellosis

☐ Goatpox/sheep pox

☐ Bluetongue

☐ Other (Specify)…………………………………………...

6. a) Have you experienced stillbirths in your flocks in the last 12 months?   ☐ Yes   ☐ No

b) If yes, number of stillbirths in:

(i) Sheep……………………   ☐

(ii) Goats………………….

Risk eating habits in humans:

☐   ☐

1. a) Do you slaughter sheep and goats at home for consumption?   Yes   No

   b) If yes, how often?

   ☐ Always

   ☐ Occasionally

   ☐ Never

2. How often do you drink raw sheep/goat milk?

   ☐ Always

   ☐ Occasionally

   ☐ Never

3. How often do you make fermented milk from raw milk?

   ☐ Always

   ☐ Occasionally

   ☐ Never

4. How often do you eat raw sheep/goat meat?

   ☐ Always

   ☐ Occasionally

   ☐ Never

4. How often do you take raw blood from sheep/goat?

   ☐ Always

   ☐ Occasionally

   ☐ Never

Disease knowledge

1. a) Are you aware of any disease, which can be transmitted from sheep and goats to human?   Yes ☐   No ☐

 b) If yes, mention the disease (s)………………………………………………………

2. a) Are you aware of diseases, which can be transmitted through sheep and goats products to human?   Yes ☐   No ☐

 b) If yes, mention the disease (s)…………………………………………………………...

3. What disease control measures do you undertake at the farm?

☐ Boiling milk

☐ Cooking meat thoroughly

☐ Vaccination

☐ Tick control

☐ Isolation of the sick

☐ Treatment of the sick

☐ Wearing gloves

APPENDIX 2: Consent form

Consent form:

Organization: University of Nairobi (Kenya) and Royal Veterinary College (United Kingdom)

Project funded by the National Research Foundation (Kenya) and the Biotechnology and Biological Sciences Research Council (United Kingdom)

Part I: Information Sheet

Introduction

I am _____, working for the University of Nairobi. I am doing a research in shoats (sheep and goats) in Kajiado.

I am going to give you information and invite you to be part of this research. Before you decide, you can talk to anyone you feel comfortable with about the research.

This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them to me.

Purpose of the research

Effective reproduction of sheep and goats allow you to produce animals for food production, but also to ensure that you have new young animals each year to replace your old reproductive livestock (mothers). However, different challenges can interfere with the capacity of sheep and goats to get

pregnant or to deliver a healthy newborn animal.

These may have important consequences to you, such as not having enough offspring to sell or maintain your flock. For this reason, it is essential to identify those factors that may have a negative or positive impact on reproduction of your animals and to estimate how much of an impact these may have.

Type of Research Intervention

This research will involve your participation by doing a questionnaire that will take one hour Following that, we will take some blood samples from 12 animals (6 ewes and 6 does); these samples will be shipped to the laboratory of the University of Nairobi to identify potential infectious diseases present in your animals. The blood sampling procedure will take around one hour. With your permission, we may also take some pictures of your animals and your farm, to be used for project purpose only, as part reports and presentations of results.

Participant Selection

You are being invited to take part in this research because we feel you are representative of the pastoralists system in Kajiado county and your answers can contribute much to our understanding and knowledge of sheep and goat reproduction and management.

Confidentiality

We will not be sharing information about you to anyone outside of the research team. The information that we collect from this research project is confidential, and no one else except the research team will access to the information documented during the interview. Any information about you will have a number   instead of your name and only the researchers will know what your number is. The data will be collected on paper and then, stored in a tablet. Both will be kept within secure, key-code access offices. All the digital data generated from the tablet will be stored on individual computer hard drives with restricted access.

Sharing the Results

We will communicate the results from the samples collected to you over the phone. If you wish us to do this, we will need your contact details. You can decide also whether the results of the laboratory test will be communicated to the veterinary officers so they can help you implement the necessary measures to protect your family and your flock. Please note that nothing that you tell us today during the interview will be attributed to you by name.

Voluntary Participation and Right to Refuse or Withdraw

Your participation in this research is voluntary, it is your choice whether to participate or not. You may change your mind later and stop participating even if you agreed earlier. You may stop participating in the interview at any time that you wish. I will give you an opportunity at the end of the interview to review your remarks, and you can ask to modify or remove portions of those, if you do not agree with my notes or if I did not understand you correctly. The questions are not going to be about any sensitive or personal issues. However, you do not have to answer any question if talking about it makes you feel uncomfortable.

Benefits

You will not be provided any incentive to take part in the research and there will be not direct benefit to you, but your participation is likely to help us find out how to improve reproduction of your sheep and goats. The results will provide an indication of the range of reproduction performance of small ruminants, which may allow you to compare your performance with other pastoralists and determine needs for improvement of your herd. The results will be communicated to the veterinary officers, who will be able to communicate these to you.

This proposal has been approved by Directorate of the Veterinary Services of the Republic of Kenya. It has also been reviewed by the Ethics Review Committee of the International Livestock Research Institute (ILRI), which is supporting the study.

Questions to elucidate understanding:

Do you know why we are asking you to take part in this study?

Do you know what the study is about?

Do you know that you do not have to take part in this research study, if you do not wish to?

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

Part II: Certificate of Consent

I have been invited to participate in research about reproductive performance of sheet and goats in Kajiado. I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked and I have been answered to my satisfaction. I consent voluntarily to be a participant in this study and I understand that the following will be done (tick as appropriate):

An interview about reproductive performance in the small ruminant flock and challenges ☐

A blood sampling procedure in 6 ewes and 6 does of the flock ☐

I also give you permission to take some pictures of the animals and the farm ☐

I want that the results of the laboratory tests will be communicate to the Veterinary Officers, so they can implement appropriate measures to protect my family and my flock. **Yes** ☐ **No** ☐

Print Name of Participant_____

Date _____

    Day/month/year

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ *(Animal Health Worker of Kajiado county)*

Signature of witness _____

Date _____

    Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands what is going to be done.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability.

I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily. A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

APPENDIX 3: Guidelines for sampling of animals:

Sample size

6 ewes and 6 does or a different number of ewes of does depending in the flock composition with a maximum number of 12 shoats (reproductive females) per flock will be sampled.

Selection of animals

Those animals with a **previous history of abortions**, **difficult births** or **problems in conceiving** will be targeted. So it is advisable to talk to the pastoralist and the animal health worker to detect those animals before collecting the samples in order to identify them. Sick animals will be included, as they will be the ones most likely to be infected by the pathogens investigated.

Animals that are in a very late stage of pregnancy and to which the sampling may cause issues will be **excluded**.

Systematic random sampling should be employed for the selection of the remaining breeding females. Every 3ʳᵈ reproductive female will be selected until the total number required for each species will be reached.

Coding samples

It is mandatory to code samples with a unique identifier using the same system across wards so that it is possible for anyone to identify, analyse the samples and communicate the result easily.

The code used will be:

Ward code/Flock ID/sample in the flock

Ward coding

- Ildamat will be coded as **I**

- Matapato South will be coded as **MS**

- Kaputiei North will be coded as **KN**

- Kenyawa-Poka will be coded as **KP**

- Magadi will be coded as **M**

Flock ID

It is the **unique identification number** for each flock. This number will be included in the questionnaire as well.

Sample in the flock

In each flock, animals included in the survey are numbered **1-12**. Sheep will be codified as **S**, while goats will be codified as **G**

Coding of special samples (animals with reproductive failures or sick animals)

D will be written at the beginning of the code.

The first letter is **D** for diseased, then ward code, flock ID and animal code will be included following the explanations provided above.

Examples:

The 1st animal is a healthy ewe; it was sampled in the flock ID 123 in Ildamat: label **I/123/1S**

The 10th animal is a healthy goat; it was sampled in the flock ID 201 in Matapato South: label **MS/201/10G**

The 6th animal is a sick sheep; it was sampled in the flock ID 56 in Magadi: **label D/M/56/6S**

Data collection sheet:

It is possible that blood sampling and interview will be carried out on different dates so a blood sampling data collection sheet has been created to be filled out during the blood sampling procedure. This sheet has to be given to the enumerators so they can introduce the data in the

questionnaire using the tablet. Date, Flock ID, Ward, Sample ID, Species and Body Condition Score (BCS).

Body condition of sampled animals

The body condition of the animal that are going to be sampled will be recorded as an indicator of health and nutritional status. A body condition score (BCS), from 1 to 5, is suggested with the following definitions taken from Ockert (Ockert, 2015):

BCS 1.0 = The goat/sheep is visually emaciated and weak. The backbone is highly visible and forms a continuous ridge. The flank is hollow and ribs are clearly visible. There is no fat cover and fingers can easily penetrate into the intercostal spaces.

BCS 2.0 = The goat's/sheep's backbone is still visible with a continuous ridge. Some ribs can be seen and there is a small amount of fat cover. Ribs are still felt and intercostal spaces are smooth, but can still be penetrated.

BCS 3.0 = The backbone is not prominent, ribs are barely discernible and an even layer of fat covers the ribs. Intercostal spaces are felt using pressure.

BCS 4.0 = The backbone and ribs cannot be seen. The side of the animal is sleek in appearance.

BCS 5.0 = The backbone is buried in fat and the ribs are not visible. The rib cage is covered with excessive fat.

APPENDIX 4: Blood sampling data collection.

Blood sampling data collection sheet

Date: _____/_____ /_____

Flock ID: _____

Ward:

	Ildamat (Kajiado Central)
	Matapato South (Kajiado Central)
	Kaputiei North (Kajiado East)
	Kenyawa-Poka (Kajiado East)
	Magadi (Kajiado West)

Sample ID	Species		BCS					Health status	
1	Ewe	Doe	1	2	3	4	5	S	H
2	Ewe	Doe	1	2	3	4	5	S	H
3	Ewe	Doe	1	2	3	4	5	S	H
4	Ewe	Doe	1	2	3	4	5	S	H
5	Ewe	Doe	1	2	3	4	5	S	H
6	Ewe	Doe	1	2	3	4	5	S	H
7	Ewe	Doe	1	2	3	4	5	S	H
8	Ewe	Doe	1	2	3	4	5	S	H
9	Ewe	Doe	1	2	3	4	5	S	H
10	Ewe	Doe	1	2	3	4	5	S	H
11	Ewe	Doe	1	2	3	4	5	S	H
12	Ewe	Doe	1	2	3	4	5	S	H

APPENDIX 5: Flock ID:

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100
101	102	103	104	105	106	107	108	109	110
111	112	113	114	115	116	117	118	119	120
121	122	123	124	125	126	127	128	129	130
131	132	133	134	135	136	137	138	139	140
141	142	143	144	145	146	147	148	149	150
151	152	153	154	155	156	157	158	159	160
161	162	163	164	165	166	167	168	169	170
171	172	173	174	175	176	177	178	179	180
181	182	183	184	185	186	187	188	189	190
191	192	193	194	195	196	197	198	199	200

When replying please quote:

REF: KJDCOUNTY / VET-GEN/VOL 1/1/393   11ᵗʰ April, 2019

University of Nairobi
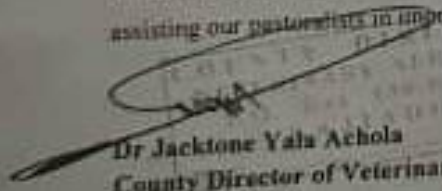
College of Agriculture and Veterinary sciences

Faculty of Veterinary Medicine

BP. O. box 29053, Kabete, Nairobi

(Att: Prof James Mbaria, Department of Public Health, Pharmacology & toxicology)

<u>RE: BBSRC funded project on enhancing ruminant livestock productivity in Kajiado County</u>

Reference is made to your unreferenced letter dated 8ᵗʰ April 2019 on the above. This is to confirm that the County has no objection on you undertaking the project in Kajiado County since the expected results of the project would have beneficial effects for the County. You are however expected to follow the necessary procedures and rules of practices applicable to all scientific research activities. You will have to work with our technical staff based at your preferred area of the project activity during the research period to facilitate easy access to the pastoralists' homesteads. It is my hope that the results from the research activity will be shared with County Government through the Department of Veterinary Services to assist in the development of livestock-related policies in regard to livestock productivity in the County. On behalf of the County Government of Kajiado, I want to register my appreciation for having chosen Kajiado County to pursue this research activity that will go a long way in assisting our pastoralists in improving the livestock productivity in the County.

Dr Jacktone Yala Achola

County Director of Veterinary Services

Kajiado County

Cc

    Chief Officer

    Agriculture, Livestock & Fisheries

    Kajiado County

Department of Public Health
Pharmacology and Toxicology
P.O. Box 29053,
Kabete, KENYA
publichealth@uonbi.ac.ke

Telephone Nairobi 020-631113
Telegraph Univet, Nairobi
Telex: 2ENIVARSITYK
Email dept.

April 8, 2019

The County Director of Veterinary Services
P. O. BOX 11-01100
KAJIADO

Dear Sir,

Ref: Introducing a BBSRC-NRF funded project on enhancing ruminant livestock productivity in Kajiado

I have the pleasure of introducing to you the above mentioned research project whose main objective is to investigate sheep and goats reproduction efficiency, challenges and mitigation strategies in Kajiado, Kenya. The project is jointly funded by the National Research Fund Kenya and the BBSRC of United Kingdom. The project is being implemented by the University of Nairobi, College of Agriculture and Veterinary Sciences through the department of Public Health Pharmacology and Toxicology and the Royal Veterinary College University of London. Broadly, the project aims at establishing the knowledge on current level of the reproduction performance indicators for different sheep and goat flocks, which could be used as a reference to identify pastoralists who are more at risk of losing their livestock and also those who are better off. This will allow us to identify the effectiveness of adaptation strategies practised that can be benchmarked for those flocks which are worse off.

The main objectives of the proposed research are: (1) To generate baseline data on Reproduction performance indicators (RPis) in small ruminant flocks in Kajiado, Kenya, and

explore how they change during shock periods; (2) To assess small ruminant pastoralists' perceptions of main reproduction challenges and mitigation strategies, and quantify their impact on RPIs; (3) To estimate the prevalence of *Brucella* spp, *Leptospira interrogans*, *Chlamydia* spp, *Toxoplasma* spp and *Coxiella burnetti* affecting small ruminants and assess their impact on RPIs; (4) To determine farmers' information and advice systems for reproduction management; and (5) To estimate financial impact of factors affecting RPIs and assess the benefits of potential mitigation strategies.
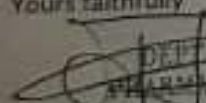
The researchers working in this project are:

- Dr. Joshua Onono (Principal Investigator at University of Nairobi)
- Dr. Garbieri Aboge (University of Nairobi)
- Dr. Pablo Alarcon (Principal Investigator at the Royal Veterinary College, UK)
- Dr. Cristina Ballesteros (Research assistant at the Royal Veterinary College, UK)
- Two MSc students at University of Nairobi
- Charlotte Armsby (MSc student at the Royal Veterinary College)

Please note that ethical approval from our institutions and the International Livestock Research Institute (recognize by NACOSTI) will be obtain prior conducting the main field work activities.

We would be happy to enlist the participation of your office to support this research project; which aims to contribute to the wellbeing of the pastoralists in Kajiado. The participation of your office would be important for us reaching the pastoralists homesteads and also in obtaining appropriate biological samples for achievement of the research objectives. We look forward to working with you and your staff to meet the objectives of this research.

Yours faithfully,

CHAIRMAN
DEPT OF PUBLIC HEALTH
PHARMACOLOGY & TOXICOLOGY

Prof. James M. Mbaria
Chairman
Department of Public Health Pharmacology and Toxicology