SEROLOGICAL AND MOLECULAR DETECTION OF BRUCELLOSIS AND ITS RISK FACTORS FOR SHEEP AND GOAT FLOCKS IN KAJIADO COUNTY, KENYA

A thesis submitted in partial fulfillment of requirements for award of Master of Science in Veterinary Epidemiology and Economics degree of the University of Nairobi

AMOS SIBUTI MWASI (BVM)

Department of Public Health, Pharmacology and Toxicology (PHPT),

Faculty of Veterinary Medicine,

University of Nairobi

2021

i

DECLARATION

I declare that this thesis is my original work and has not been submitted for the award of a degree in any other university or institution of higher learning. No part of this thesis shall be reproduced without the authority of the author or /and the University of Nairobi.

Signature…………………………………Date………3rd November 2021…………….

Amos Sibuti Mwasi (BVM).

SUPERVISOR APPROVAL

This thesis is submitted for examination with my approval as University supervisor:

Dr. Joshua Orungo Onono (BVM, MSc, MBA, Ph.D.)

Signature: ……………………………. Date: …3/11/2021…………….

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi

Dr. Gabriel Oluga Aboge (BVM, MSc, Ph.D.)

Signature: ……………….…..............Date: …4/11/2021............

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi

Dr. Pablo Alarcon Lopez (BVM, MSc, Ph.D.)

Signature: Date: 3/11/2021

Royal Veterinary College, University of London

DEDICATION

This is to my loving parents William Mwasi and Lucy Baru. It is because of their selfless efforts that I had the opportunity to work on this thesis. This also goes to my dear wife Caren Ghati and my daughter Shirleen Bhoke. I cannot repay the ample time and peace of mind they gave me to work on this great project. Their encouragement was worthwhile. I believe we shall achieve much more together.

ACKNOWLEDGEMENT

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

BBSRC   **-**   Biotechnology and biological sciences research council

WHO   -   World Health Organization

RBT   -   Rose Bengal Test

AST   -   Allergic skin test

BLAST   -   Basic local alignment search tool

CFT   -   Complement fixation test

bp   -   Base pair

cELISA   -   Competitive Enzyme linked immuno-sorbent assay

MAT   -   Microtiter Plate Agglutination Test

ILRI   -   International livestock research institute

MRT   -   Milk ring test

DNA   -   Deoxyribonucleic acid

STAT   -   Standard Tube Agglutination Test

OD   -   Optical density

SPSS   -   Statistical Package for Social Sciences

ODK   -   Open data kit

SATT   -   Serum Agglutination Tube Test

dNTP   -   Deoxynucleotide triphosphate

EDTA - Ethylenediaminetetraacetic acid

CDC - Center for disease control.

PCR - Polymerase chain reaction

FAO - Food and Agriculture Organization of the United Nations

ABSTRACT

Brucellosis is a zoonotic disease that poses significant public health risk globally. This study was done to estimate molecular and serological prevalence of Brucella pathogens in sheep and goats in five wards (Matapato South, Kaputei North, Ildamat, Kenyawa Poka and Ilodokilani) in Kajiado County. Samples of serum and whole blood were randomly collected from 1,560 sheep and goats in Kajiado County and screened using RBT. Additionally, questionnaires were administered to collect flock data on risk factors associated with Brucella infections in sheep and goats. A total of 130 pastoralists were interviewed. A total of 320 serum samples were selected based on history of abortion and presence of sick ewes and does in flocks including those samples that tested positive on RBT, which were then tested using cELISA for presence of *Brucella melitensis* and *Brucella ovis* antibodies. About 72 whole blood samples drawn from animals that tested positive on serological tests (RBT and C-Elisa) and other suspected serological negative test samples with history of abortions, were further subjected to conventional polymerase chain reaction (PCR) using *B. Melitensis* and *B. ovis* specific primers to test for presence of IS711 and omp31 (JX081250.1) genes.

The overall flock seroprevalence of Brucellosis in sheep and goats in Kajiado County based on RBT was 5.38% (7/130). The flock seroprevalence in sheep was 2.31% (3/130) and 3.85% (5/130) for goats. The flock seroprevalence in sheep was 8 % (2/25) in Matapato south, but there were no positive cases by RBT in sheep from Ildaamat, Kenyawapoka, Kaputiei north and Iloodokilani wards. In goats, flock seroprevalence was 8 % (2/25) in Matapato south, 9.52% (2/21) in Kenyawapoka and 3.70% (1/27) in Ildaamat ward. Two serum samples tested positive for *Brucella melitensis* by cELISA. One of these samples, which was from a goat, had also tested positive by RBT. Both samples were from Matapato south administrative ward. The overall flock

molecular prevalence for Brucella in the sheep and goats in Kajiado County by conventional PCR was 24.49% (12/49). All the positive samples by conventional PCR were from goats. The flock molecular prevalence in goats in Kajiado County was therefore estimated at 35.29% (12/34). The flock molecular prevalence in goats was 50 % (4/8) in Matapato south, 38.46% (5/13) in Kenyawapoka, 33.33% (2/6) in Ildaamat ward and 14.29% (1/7) in Kaputiei north.

Eight variables were analyzed at flock level by logistic regression with flock Brucella infection status as the outcome of which six were found to be significantly associated with flock Brucella positivity either as risk factors or protective factors.These included experiencing abortion in sheep and goats flock (odds ratio (OR) = 3.09; 95% CI), introduction of new animals into the flock (OR = 1.33; 95% CI), mixing with other flocks in communal grazing fields and common watering points (OR = 1.56; 95% CI), sharing male animals with other flocks for breeding purposes (OR = 1.03; 95% CI), knowledge of brucellosis and other zoonotic diseases (OR = 0.85; 95% CI) and seeking of veterinary services (OR = 0.23; 95% CI). Detection of *Brucella melitensis* and *Brucella ovis* DNA and antibodies against *Brucella melitensis* from the collected samples confirms the presence of brucellosis in sheep and goats in Kajiado County.

CHAPTER ONE: INTRODUCTION

1.0 BACKGROUND

Sheep and goats farming are a significant component of livestock production systems in developing countries, especially within Africa (Nyariki and Amwata, 2019). These livestock are mainly kept for sale to obtain quick money to pay for household needs, besides they are a ready source of household food in form of milk and meat, manure, while some families keep them for purpose of prestige (Kelly et Al., 2017). Furthermore, they are easier to manage as compared to cattle by smallholder pastoralists' families due to lower animal feed requirements and therefore costs, quicker turnover and easier management and greater tolerance to less favorable climatic conditions (Franc et Al., 2018).

The population of sheep and goats in Kenya is currently estimated at 17 million sheep and 25.8 million goats, of which 57% and 50%, respectively, are considered pastoral based systems (KNBS, 2019). The population is higher than that for cattle raised within the pastoral areas in Kenya. Furthermore, it is projected that the demand for meat from small ruminants will increase by up to 46 % by the year 2050 (FAO, 2017, Nyariki and Amwata 2019).

Despite the significance of sheep and goats in the livelihoods of pastoralists, their productivity remains low, which has mainly been blamed on presence of diseases, poor management and poor and low nutrition (Blasco and Molina 2011). Brucellosis is among the predominant infectious and zoonotic diseases considered as constraints for sheep and goats productivity within pastoral systems. The economic losses from these infections stem from breeding inefficiency, loss of offspring, reduced meat and milk production as well as restriction on animal movement and export of sheep and goats and their products to the international markets (Tewodros and Dawit, 2015).

The main clinical signs of brucella infection are abortion, mastitis, orchitis and epididymitis which results in sterility, due to the localization of the *brucella* pathogens in the reproductive organs (Amjadi et al., 2019). The shedding of *Brucella* organisms by sheep and goats through udder secretions and semen, and in poorly disposed placentas from infected animals has been documented in previous studies (Poester *et al*., 2013). Brucellosis can also be transmitted between animals by direct contact with infected excretions and tissues, consumption of milk, venereal, through the conjunctiva or through inhalation of pathogens in aerosols (Alemneh & Akeberegn, 2018). Serological techniques have been widely involved in brucella diagnosis in small ruminants in the last decade (Tewodros 2015, Kelkay *et al*., 2017), and it has been reported that a combination of serological and molecular methods may provide reliable approach for the detection and diagnosis of Brucella infections in animals.

1.1 PROBLEM STATEMENT

Brucellosis is among the most severe causes of reproductive losses in sheep and goats. It causes infertility, abortion, and neonatal mortality (Ruiz-Ranwez *et al*., 2013). additionally, it is zoonotic and pastoralists can get exposed to infections through consumption of raw meat, milk and blood and touching infected placenta and aborted material without wearing protective attire (Onono *et. al*., 2019).

The disease has detrimental effects on people and animals alike. Conservative cultural practices enhance progress of the disease especially in developing countries, limiting their economic growth and development by acting as barriers to international markets. The disease causes a decline in the productivity in sheep and goats that compromises food security and result in profound emotional suffering to pastoralists whose flocks are affected. Besides, the burden it has on infected humans through the human disability-adjusted life years which have been reported in farming systems across Africa (Charypkhan *et al*., 2019). The costs are associated with high cost of diagnosis and medical treatment, loss of working days and deterioration of the socioeconomic status of infected individuals(Franc et al., 2018). Routine diagnostic methods for Brucella infections in Kenya majorly rely on detecting antibodies specific to Brucella lipopolysaccharide (LPS) specific. However, there can be false positive results due to antigenic similarities that exist between Brucella LPS and other bacteria like *E. coli*, some Salmonella and *Yersinia*. These serodiagnostic tests are also time-consuming, have low sensitivity and place laboratory technicians at a significant risk of infection (Zakaria, 2018**)**. A combination of serological tests for antibodies against the pathogen and detecting DNA is necessary for a conclusive diagnosis of Brucellosis to provide solid evidence of the presence of disease (Akoko *et al*., 2020). This study

sought to detect brucella in sheep and goat flocks in Kajiado and identify the potential exposure factors for infection of flocks.

1.2 OBJECTIVES

1.2.1 Overall objective

To investigate the prevalence of Brucellosis in sheep and goats and associated risk factors in Kajiado County.

1.2.2 Specific Objectives

1. To determine the prevalence of *Brucella* infections in sheep and goats in Kajiado County.

2. To determine the risk factors associated with Brucellosis in sheep and goat flocks in Kajiado County.

1.3 Research Questions

1. Which Brucella species are present in sheep and goats raised in Kajiado County?

2. What are the flock level predisposing factors associated with Brucellosis in sheep and goats in Kajiado County?

1.3 JUSTIFICATION OF THE STUDY

Available data on molecular epidemiology of ovine and caprine brucellosis in Kenya is scanty, with little up to date research. The only documented reports are serological surveys that recorded the presence of Brucella antibodies from sheep and goats among the pastoral communities. *Brucella melitensis* is one of the most pathogenic zoonotic Brucella species. Despite this fact, the sheep and goat industry continues to grow rapidly in Kenya, with the growth rate predicted to increase by 180% by the year 2030. Therefore, there is need to accurately establish the flock prevalence the disease in small ruminants, especially in pastoral regions of the country in order to manage it properly.

RBT is recommended by OIE for brucella screening. Despite sensitivity of RBT being high, it has a relatively low specificity may give false-positive results and requires confirmation by more specific tests. Other serological tests such as ELISA also suffer low sensitivity in brucella diagnosis in sheep and goats. These limitations of serological tests of brucellosis make them not to be ideal for diagnosis. The application of molecular tests to complement serological tests in the current study will significantly advance the understanding of host-pathogen interactions and provide accurate and reliable detection of Brucella in the sheep and goats of Kajiado County.The findings of this study will pave way for an understanding on the link between sheep and goats and human infection by Brucella pathogens.

The recommendations from this study will enable policy makers and governments to properly plan and develop programs for prevention and control of brucellosis in small ruminant populations as part of controlling human infections. The use of an interdisciplinary one health approach would eventually lead to successful elimination or even eradication of the disease.

CHAPTER TWO: LITERATURE REVIEW

2.0 Background on brucellosis in small ruminats

Brucellosis is a bacterial zoonosis that causes significant health, financial and economic challenges globally mostly affecting developing countries where pastoral communities rely entirely on livestock production as a source of livelihood. Brucellosis is also referred to as Enzootic Abortion, Ram Epididymitis, Contagious Abortion or Bang's disease in animals. It has also been described as Malta fever, Gastric Fever, Undulant Fever or Mediterranean fever in people (Alemneh & Akeberegn, 2018). It is a significant cause of reproductive losses in livestock. Symptoms of infection include third trimester abortions, placentitis, epididymitis, orchitis, still births and birth of weak offspring. Animals that experience abortion may have retained afterbirth (Dean *et al*., 2012). In the lactating animals brucellosis would cause reduced milk production and sub-clinical mastitis, while abortions are reported in pregnant animals (Franc *et al*., 2018). In addition, Brucellosis has significant public health implications and if transmitted to people it can cause acute fever and even chronic illness (Bodenham, 2020). The people at a higher risk of Brucella infection are individuals who are frequently in contact with animals like veterinarians, abattoir workers, and laboratory workers.

2.1 Causes of brucellosis

It is caused by *Brucella* organisms which have been demonstrated microscopically as short rods, small cocci or cocco-bacilli, measuring about 0.5 - 0.7 by 0.6 - 1.5 pm in size. They have been described as non-encapsulated, non-sporulating and non-acid-fast bacteria which stain gram-negative. *Brucella* organisms are categorised into six main species; *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae* (Bang, 1897, Meyer and Shaw 1920). Brucellosis in

sheep and goats is caused by *B. ovis* and *B. melitensis*. *B. melitensis* are the most pathogenic and virulent species in people (Poester *et al*., 2013). Most brucella strains thrive in anaerobic environment with optimal pH of 6.6 to 7.4 and temperature of 37^0C. Brucella pathogens lose viability at 56^0C (Tegegn & Feleke, 2016).

The *Brucella* species do not have strict host preference. They may infect secondary hosts but such infections appear to be self-limiting (Blair *et al*., 2003). *Brucella* pathogens primarily target the reproductive organs. Transmission to other hosts especially wild animals can be through exposure to contaminated carcasses or other contaminants (Wyatt, 2005).

Brucella species are mainly found in their primary hosts, but cross infection can occur and the severity of infection varies from one host to the other (Alton *et al*., 1998, Eisenberg et al., 2012).

2.2 Occurrence of ovine and caprine Brucellosis globally

Small ruminant brucellosis occurs worldwide. The disease spreads to humans and result in major economic challenges in developing countries especially Africa (Mustafa et al., 1995). This is due to poor practices of importing exotic breeds of sheep and goats without proper veterinary inspection. However, it was declared eradicated by OIE in some developed countries in Europe (Corbel, 2006). This was achieved through rigorous implementation of one health disease control measures involving vaccination and elimination of infected animals (Kelkay *et al*., 2017). But, some developed countries have faced challenges in their effort to eradicate the disease since Brucella pathogens are able to infect multiple species and they can survive for long periods in feral populations of ruminants and swine as reservoirs (Berger, 2016). For example, America invested heavily in a national programme on disease control efforts, and they managed to reduce bovine brucellosis prevalence by about 12 %. However, elk and bison which are reservoirs for

Brucella pathogens made it impossible to completely eradicate the disease from the country (Pedersen *et al*., 2014). Brucellosis can be transmitted among people, livestock and wildlife especially in pastoral communities in developing countries (Assenga *et al*., 2014).

A previous study of human brucellosis was done using data from 15 countries across the world. A strict exclusion criteria was used to compile scientific papers published between 1990 and 2010 and found that many countries had poor health systems for Brucella diagnosis, which could probably underestimate the disease burden. Brucellosis prevalence in the developed regions was lower compared to that in developing countries. However, Brucellosis still remains of significant public health concern globally (Dean *et al*., 2012).

2.3 Occurrence of ovine and caprine Brucellosis in East African region

Brucellosis was officially reported through laboratory diagnosis in Kenya for the first time in the 1930s. According to veterinary epidemiology and economics unit (VEEU) and the zoonotic disease unit (ZDU), the disease has since become one of the most significant livestock diseases in Kenya (FAO, 2017).A review of brucellosis in Kenya in the year 2016 revealed that there was bacteriological evidence of brucellosis in livestock and people but there was no evidence in small ruminants in the country at that time (Njeru *et al*., 2016). Another survey has previously reported a prevalence of 8.6% (6/69) in sheep and 7.3% (5/67) in goats in Kajiado where 167 sheep and 167 goats were screened for Brucellosis using RBT and cELISA tests (Nakeel *et al*., 2016). A study was conducted in Marsabit where a total of 227 households and their livestock were screened by ELISA test and the prevalence of Brucellosis was found to be 16.09% in goats and 11.89% in sheep (Kahariri, 2018). In another study done at Garissa, sera from sheep and goats were tested using RBT and sero-positive samples further subjected to CFT using serial interpretation, and a sample was only classified to be positive when both tests results turned

positive. According to this study, the overall sero-prevalence for brucellosis at individual animal-level was 20.0%, 24.3% in goats and 12.5% in sheep (Obonyo, 2018). A hospital based study in Garissa County among febrile patients, reported a Brucellosis sero-prevalence of 31.8% and a molecular prevalence of 15.4% (Kiambi, 2014).

In another study conducted in Narok and Marsabit Counties, a brucella prevalence of 33.3% in livestock and 38.5% in people was found. Cattle and camels had a higher prevalence of *B. abortus* while sheep and goats were found to have a higher prevalence of *B. melitensis* (Akoko et al., 2021).

In a study to establish the serological and molecular prevalence of brucellosis in people, wildlife and livestock in one of the pastoral regions of Tanzania, RBT and ELISA tests were used and a brucellosis seroprevalence of 1.6 % was found in goats and 0.6 % in humans. Multiplex PCR detected *Brucella abortus* bovine milk but did not detect any brucella antigens in goat milk (Assenga *et al*., 2014).

2.4 Transmission and pathogenesis of brucellosis among sheep, goats and humans

Sheep and goats are commonly infected through ingestion of contaminated feed and water, inhalation during overcrowding, direct contact through intact skin and conjunctiva and by suckling milk from an infected dam. Transmission may also occur through artificial insemination, using male animals for breeding without proper medical examinations, exposure to aborted materials, contaminated placenta and postpartum discharge from an infected female animal (Njeru *et al*., 2016).

The brucella pathogens mainly inhabit organs with numerous reticuloendothelial cells (karim *et al*., 2019). The pathogen replicates intracellular and results in hepatomegaly and splenomegaly in

humans (Von Bargen *et al*., 2015). Brucella pathogens multiply predorminantly in genital organs of their hosts. The placental conditions favour multiplication of brucella organisms leading to abortion, which in turn spreads the Brucella progeny to new hosts (Letesson *et al*., 2017).

 The uterus and epididymis have high concentrations of erythritol which provides energy for Brucella, enhancing their growth (Rodríguez *et al*., 2012). The progesterone produced by sheep and goat placenta has been shown to enhance the growth and development of the brucella organism (Amjadi *et al*., 2019).

2.5 Detection of Brucella infection in livestock

Diagnosis and control of brucellosis in livestock must be done at flock level in order to increase the chances of detecting and effectively managing it. The disease can have a long incubation period and individuals may not develop antibodies against it hence giving false negative serological results. Detection of the disease in at least one animal provides sufficient evidence of the disease in the flock and implies that the animals that test negative could be at the incubation stage but can infect other animals and humans hence posing a great risk (Tewodros and Dawit, 2015). The diagnostic tests routinely used for brucella can be categorized into two: detection of immune response to the antigen especially production of specific antibodies against the pathogen and detection of the presence of brucella pathogens in the host (Mohseni *et al*., 2017).

The tests that are commonly used to detect an immune response to the brucella antigens include the MRT, RBT, STAT, MAT) and ELISA (Khan et al., 2018). MRT is used to screen for brucellosis in dairy animals but has serious limitations in sheep and goats since it can only be used on milking animals. CFT is a good test for active infection because it can single out IgG antibodies that are usually during infection (Musallam *et al*., 2016). RBT is valuable in the rapid

screening for ovine and caprine brucellosis. It has a high sensitivity (>99%) but relatively low specificity which can result in false-positive results. It has a low positive predictive value and therefore requires confirmation by more specific tests (Neharika et al.,2018). ELISA can be used for screening of sheep and goat flocks for brucellosis. It can be used together with other tests for better diagnosis of infected animals (Gutiérrez-Jiménez et al., 2019).

However, specific antibodies detection or observing a hypersensitivity reaction in an individual gives only a tentative diagnosis, but is mostly the only feasible and economical diagnosis available in field conditions (Mohseni *et al*., 2017). Factors like vaccination can result in false positive results when serological tests are used and one must bear this in mind when interpreting those results. Dermal hypersensitivity reactions also only indicates that the individual was previously exposed to the brucella pathogens or vaccinated, but not necessarily an evidence of active infection (WHO, 2016).

The culture and direct demonstration of the causal organism can be done microscopically with staining examination, fluorescence, culture on special nutrients and in animal experiments with guinea pigs to provide evidence of infection. Smears can be made from vaginal swabs, aborted fetuses, blood or milk and stained with stamps method. Necropsy samples can be obtained from the spleen or lymph nodes. This Confirmation of the pathogen is done on appropriate culture and selective media (Yasmin et al., 2018). However, some infected individuals may not grow a positive culture upon incubation and the required facilities and safety levels for culture are not always easily accessible (Musallam *et al*., 2016). The achievement of a reliable diagnosis of brucellosis through culture and isolation is therefore a tedious process since isolation is affected by factors such as the fastidious nature of Brucella, a lesser number of viable pathogens in the

available sample, delay in the sample submission resulting in putrefaction and a prolonged

incubation period may lead to a failure in its isolation (Hanci *et al*., 2017).

2.5.1 Molecular diagnosis of ovine and caprine Brucellosis

Detecting the specific genomic DNA is very useful for a conclusive diagnosis of brucella in

sheep and goats. Molecular techniques are replicable, standardisable, pose very minimal risk to

laboratory technicians and take a short amount of time to carry out. The molecular signatures of

Brucella mainly rely on genomic variations of the biovars. DNA fragments are subjected to PCR

using specific oligonucleotide primers with sufficient *Taq* DNA polymerase at appropriate

annealing temperature. The primers designed for brucella spp are usually specific to the target

size of amplicon products elucidated in electrophoresis. A variety of molecular marker based

assays are currently used for confirmatory diagnosis of brucella. The molecular markers target

the conservative brucella genes that include; bcsp31, omp2, omp19, bp26 and IS711 genes.

Polymerase chain reaction and sequencing are simple, quick and highly sensitive for Brucella

detection, especially those using the 16s rRNA as targets and the bcsp31 genes (Singh *et al*.,

2014), which are highly conserved in Brucella genus (Al Dahouk ET AL., 2007)

A previous study that was done in Gerais, Brazil combined serological and molecular tests to

detect brucellosis in small ruminants. Samples were subjected to AGID, ELISA, RBT and PCR

tests. The study found 4% (2/50) positive by PCR. They did not find any positive samples in

RBT, implying that there were no antibodies against *Brucella melitensis*. This confirmed that the

samples detected by ELISA were *B. ovis* (Costa *et al*., 2016).

Another study sought to find brucellosis prevalence in Bangladesh using serological and

molecular diagnostic tools, a total of 40 sheep and 50 goats serum samples were collected and

screened by RBT and those that tested positive were subjected to SAT CFT, ELISA and quantitative real time PCR. All samples tested negative to RBT but 12 tested positive for *Brucella* DNA (Sarker et al., 2016).A study on caprine brucellosis was coducted in a Sultanate of Oman, where RBT, I-ELISA and CFT were coupled with PCR to detect brucellosis. An overall seroprevalence of 11.1% was found. Further confirmation using biochemical tests and PCR revealed *B. melitensis* (Yasmin *et al*., 2018).

2.6 Empirical review of studies on predisposing factors for animal and human infection

The predisposing factors for brucellosis infection in animals can be grouped into factors that facilitate the spread of the pathogens from an infected flock to a susceptible one and the factors that maintain and spread brucella infection in the flock. They can also be categorized into host, agent or extrinsic factors (Franc *et al*., 2018).

The transmission of Brucellosis from one animal to another is associated with the mixing of flocks from different regions when livestock are moved in search of pasture especially during periods of prolonged drought, grazing the animals in communal fields and when different flocks of animals gather around public watering points such as water pans, rivers and government-built boreholes (Kunda et al., 2010). It is widely accepted that kids and lambs may become infected before or soon after birth and tend to become free from infection before reaching breeding age, occasionally infection persists much longer. Brucella pathogens mostly cause disease in sexually mature animals (Kelkay *et al*., 2017).

People can contract Brucellosis in livestock by practicing risky sociocultural activities that expose them to the pathogen either by direct contact with infected animals especially when one has broken skin or consuming animal products (Mutua *et al*., 2017). People who work closely

with animals, such as veterinarians and herdsmen are at a greater risk of exposure to infection (Luce et al., 2012, Asiimwe *et al*., 2015). Abattoir workers have been shown to be at a great risk of infection especially those involved in cutting animals' throats and those that clean the offal (Swai et al., 2009). A previous research that was carried out in hospitalized patients in Kampala Uganda showed that gender (being female) and living in slums were major risk factors for brucellosis infection in people (Makita *et al*., 2011).

Another study in the northern region of Tanzania with a majority of the pastoralists showed that close interaction with animals puts people at risk of infection with brucellosis (Kunda *et al*., 2010). When pastoralists migrate with animals in search of pastures and water, these animals may get infected and in turn transmit them to people. Infection in humans and livestock was significantly associated (p<0.05) (Migisha, 2018).

2.7 Empirical review of studies on zoonotic aspects of Brucellosis

Brucellosis, especially where *B. melitensis* is enzootic in small ruminants, has been found to cause severe and chronic disease in people (Moreno, 2014). Brucellosis in people is widely distributed globally and has been found to be endemic in Africa and Asia (Demena, 2019).

Brucellosis is transmitted to people by direct contact with abortus and carcasses of infected animals handling retained afterbirths with bare hands, consumption of raw milk, meat and contaminated water (Onono *et al*., 2019). It has also been found to be one of the common laboratory acquired infections (Weinstein & Singh, 2009).

Brucellosis due to *B. melitensis* causes an acute condition with non-specific symptoms that transforms to a chronic debilitating illness in people if it is not treated (Buzgan et al., 2010). The chronic form of the disease is an insidious illness with vague signs and symptoms that can be

confused with other diseases(Franc *et al*., 2018). A previous study on brucellosis was carried out in northern Tanzania, a pastoral community with a livestock-wildlife interface. *B. melitensis* was isolated from 50.0% (7/14) of patients and *B. abortus* from 7.1% (1/14). Age and herding were identified as risk factors with young patients being more likely to have brucellosis compared to older patients. Individuals who herded livestock in the previous 12 months were also more susceptible to infection (Bodenham *et al*., 2020).

In a previous study, serum samples were obtained from human patients in north eastern Kenya and subjected to serological and molecular tests for brucellosis. 80.13% (117/146) of patients in the study tested seropositive for Brucellosis. 2.7% of seronegative patients tested positive for brucellosis by molecular test. These results highlighted the usefulness of combining serological and molecular tests in diagnosis of acute brucellosis (Njeru *et al*., 2016)

2.8 Empirical review of studies on the Impact of Brucellosis

Brucellosis causes significant economic losses globally especially in the developing world where very little research has been done to provide an account of losses (Shirima and Kunda 2016). The losses normally result from the culling of sick animals, animals lost through abortion, decreased milk yield especially when mastitis is involved, costs incurred in diagnosis and treatment of the infected animals and people, vaccination of flocks that are at risk of infection, trade restrictions and loss of market for the animals and their products especially the international market and administrative costs invested in programs to manage the disease (Singh *et al*., 2015).

Some developed countries have faced challenges in their effort to eradicate the disease. Brucella pathogens are able to infect multiple species and they can survive for long periods in feral populations as reservoirs (González-Espinoza et al., 2021). The United States invested a large amount of resources in a national program and managed to significantly decrease the prevalence

of brucellosis in the country. However, elk and bison which are reservoirs for brucella pathogens made it impossible to completely eradicate the disease from American soil (Pedersen *et al*., 2014).

Small ruminant productivity losses resulting from brucellosis are not well researched in Kenya. Human disability-adjusted life year burdens for brucellosis are significant. Brucellosis can result in chronic complications which increase the cost of treatment in delayed diagnosis. There is need to introduce earlier differential diagnosis for brucellosis in high-risk populations(Mcdermott *et al*., 2013).

2.9 Treatment remedies for brucellosis

Brucellosis can be treated by common antibiotics including tetracycline, aminoglycosides, fluoroquinolones. Most *Brucella* strains are susceptible to these groups of antibiotics. However, their susceptibility varies with the species and strain of brucella causing infection (Saxena *et al*., 2018). Subjecting brucellosis patients to long-term treatment with antibiotics has been found to help in controlling the damage caused by infection to a great extent (Solera et al., 2017).

 Combining different groups of antibiotics such as tetracyclines and aminoglycosideshas has been shown to increase the efficacy of treatment (Meng *et al*., 2018). These treatment protocols are used both in veterinary and human cases and have been proven to work well even experimentally.

2.10 Review on prevention and control of brucellosis

Controlling the disease in livestock populations is the primary step in managing brucellosis (Godfroid et al., 2013). This can be done by vaccination of livestock, culling of the sick animals, epidemiological monitoring and surveillance testing. So far there is no approved Brucella

16

vaccine in humans. However, there are three major vaccines in animals; RB51 and S19 against *B. abortus* infections, while Rev1 against *B. melitensis* (Stevens et al., 1994). Vaccination has been found to lower the likelihood of abortion and therefore breaks the cycle of transmission hence protecting the remaining livestock and humans from infection (Roth et al., 2003).

Test and culling strategy coupled with rigorous vaccination have been used to eradicate the disease in some regions (Blasco and Molina 2011). These principles however depend on the disease burden and the economic status of the country involved. The need to test and cull, introduced and resident animals likely to be carriers is recommended, but difficult to be effective because of the inaccuracy of tests and the poor economic status especially in developing countries (Musallam *et al*., 2016). The One health principle has proven essential in the management of brucellosis (Zinsstag et al., 2011, Desta, 2016).

Effective prevention and control of brucellosis comes down to individual herds where proper hygiene at parturition, separate pens for lambing and kidding need to be routine practices (Mandal *et al*., 2017). Brucella pathogens can be destroyed by most disinfectants such as formaldehyde and iodophors. They can also be destroyed by pasteurization temperatures (Makita et al., 2010).

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Study area

A cross-sectional study of 130 sheep and goat pastoralist flocks was conducted in Kajiado County, Kenya. Residents of this county mainly rely on pastoralism 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.

The study area was purposively selected based on the relatively high livestock numbers and easy accessibility. Data was collected from Kaputiei North and Kenyawa-Poka administrative wards in Kajiado east sub county, Ildamat and Matapato South administrative wards in Kajiado central sub county and Iloodokilani administrative ward in Kajiado west.

3.2 Target population

Reproductive flocks with enough females to reproduce and multiply were selected. Flocks with 10 ewes and/or does and at least 50 sheep and/or goats were selected.

3.3 Study design

We used a cross-sectional design. Serum and whole blood samples were obtained from sheep and goats to test for Brucellosis using PCR. In addition, a retrospective survey based on pastoralist interviews about their flock management practices was done.
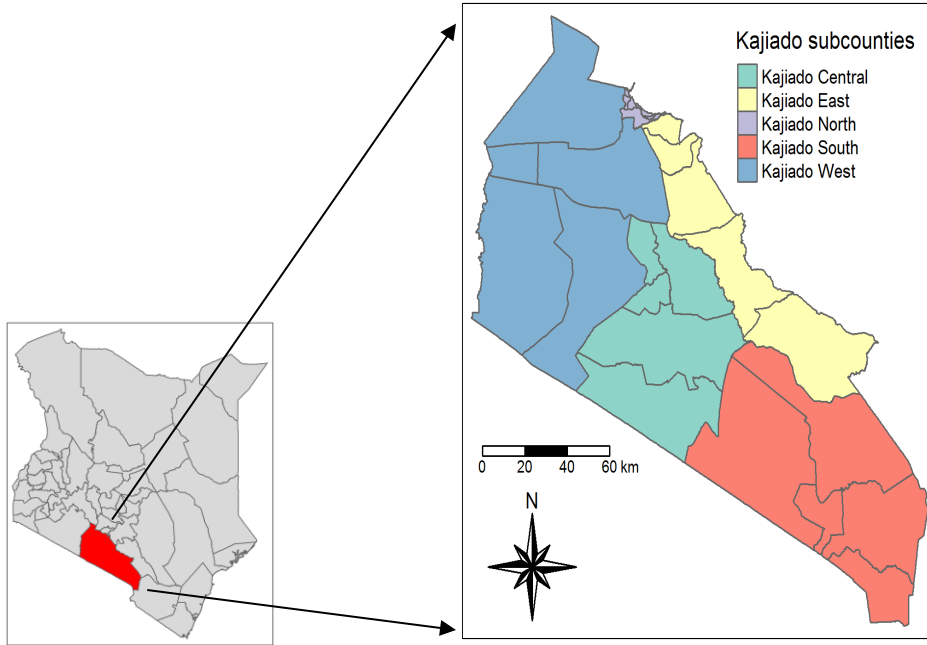
Figure 1: A map showing Kajiado County, Kenya.

3.4 Sample size

The number of flocks sampled within Kajiado County was determined by the following formula.

(Dahoo et al., 2014). $n = \frac{Z_\alpha^2 pq}{l^2}$

$n = \frac{1.962 \times 0.5 \times 0.5}{0.1^2}$, $\quad n = 96$

The obtained value was adjusted upwards by 30% and this gave a sample size of 130. Therefore, a total of 130 flocks were sampled. The following formula was then used to obtain the number of sheep and goats that were sampled in each flock (blood sampling).

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

Where: n = required sample size, α = 1 – confidence level (0.05), D = estimated minimum number of diseased animals in the group (population size x minimum expected prevalence), and N = population size. For a flock size, N of 100 small ruminants, if the disease was present at 20% prevalence or higher (D), with 90% herd sensitivity, assuming 95% test sensitivity, perfect specificity, 5% precision and 95% confidence interval (α = 0.05), assuming a design effect of 2, the required sample size, n per flock will be:

$$n = \left(1 - (0.05)^{1/20}\right)\left(100 - \frac{20 - 1}{2}\right)$$

$$n = 12$$

12 sheep and goats in each of the 130 flocks were sampled, resulting in a total of 1,560 sheep and goats.

3.5 Selection of sampling units

Pastoralist flocks were selected randomly through the identified areas, 26 pastoralists were surveyed from each ward. Female animals of reproductive age (2 years and above) within the flock were selected through systematic random sampling.

3.6 Laboratory processing

3.6.1 Rose Bengal Test

All the 1560 sheep and goat serum samples collected from Kajiado County were screened using RBT for anti-brucella antibodies. 25ul of the test serum and75ul of the RBT antigen were dropped on each well of the agglutination plate and immediately mixed thoroughly using a wooden splint and the agglutination plate rocked gently for about 2 minutes. The results were read immediately in a well-lit place and interpreted as either positive or negative. A sample was

considered positive for RBT when there was any visible agglutination at 2 minutes (Tegegn & Feleke, 2016).

3.6.2 Detection of Brucella antibodies using competitive ELISA (Apha scientific, 2014)

Reagent preparation

The diluting buffer was made by adding 5 tablets of PBS, 500ul phenol red indicator and 250ul of Tween 20 to 500ml distilled water.

Wash solution was made by adding contents of the ampoule of Na_2HPO_4 and 1ml of Tween 20 to 10 litres of distilled water.

The conjugate was made by adding 20ul of the conjugate concentrate (BM40) to 50ml of diluting buffer and kept at -20^0C ready for use.

Stopping solution was prepared by diluting the contents of the ampoule of citric acid with 38ml of distilled water.

The positive and negative control samples were reconstituted each with 1ml of sterile distilled water.

OPD was prepared in an opaque container (since it is highly sensitive to light) by dissolving one tablet of urea H_2O_2 in 12ml of distilled water to make a substrate and chromogen solution then adding the OPD tablet and mixing thoroughly.

Method

- 20ul of each serum sample was added per well, leaving columns 11 and 12 for controls.
- 20ul of the positive control was added to wells F11, F12, G11, G12, H11 and H12. 20ul of the negative control was added to wells A11, A12, B11, B12, C11 and C12.

- The remaining wells in columns 11 and 12 had no serum and acted as the conjugate control; D11, D12, E11 and E12.

- 100ul of the prepared conjugate solution was then dispensed into all wells, giving a final serum dilution of 1/6.

- The plate was vigorously shaken on the microtitre plate shaker for two minutes to mix the serum and conjugate solution.

- The plate was then covered with a lid and incubated at room temperature for 30 minutes on the shaker.

- The contents of the plate were shaken out before washing each of the plates 5 times with washing solution.

- The plate was then dried by tapping firmly onto a few layers of absorbent towel until no more liquid was removed.

- 100ul of OPD solution was added to all the wells. The plate was incubated at room temperature for 15 minutes.

- The microplate reader was switched on and allowed to stabilize for 10 minutes.

- The reaction was slowed down by adding 100ul of stopping solution to all wells.

- The Optical Density (OD) was determined using an ELISA reader (BioTek Synergy HT, BioTek Winooski, VT 05404 United States) at a wavelength of 450 nm.

Plate acceptance criteria

Lack of color formation meant that the sample had tested positive. A plate was considered valid if the mean OD of the 6 negative controls at 450 nm was greater than 0.700 and the mean OD of the 6 positive controls was less than 0.100, the mean OD of the 4 conjugate control wells is greater than 0.700 (the optimal mean conjugate control OD is 1) and the binding ratio (mean of

the 6 negative control wells divided by mean of the 6 positive control wells) is greater than 10. A cut-off was established using the conjugate control, i.e. 60% of the mean OD of the four conjugate control wells. Any OD equal to or lower than the cut-off value was considered as being positive, while values above the cut-off were considered negative.

3.6.1 Extraction of pathogens- genomic DNAs from sheep and goat-blood

The stored whole blood samples were obtained from the freezer and allowed to thaw at room temperature for about 20 minutes then genomic DNA of *Brucella* spp extracted using a commercial Thermo Scientific GeneJet Whole Blood Genomic DNA Purification Mini-Kit as follows:

- 20μl of Proteinase K solution was added to 200μl of whole blood and mixed by vortexing.

- 400ul of Lysis solution was added and mixed thoroughly by vortexing to obtain a uniform suspension.

- The sample was then incubated at 56^0C for 10 minutes while vortexing occasionally until the cells were completely lysed in the process.

- 200μl of 70% ethanol was added and the solution and mixed thoroughly by vortexing.

- The mixture was transferred to the spin column, centrifuged for 1 minute at 8000 rpm.

- The collection tube containing the flow-through solution was discarded then the column placed into a new 2ml collection tube.

- 500μl of wash Buffer WB I was added to the column and centrifuged for 1 minute at 10,000 rpm.

- The flow-through was discarded and the column placed back in the collection tube.

- 500µl of Wash Buffer WB II was added to the column and centrifuged for 3 minutes at maximum speed (14,000 rpm).

- The collection tube was emptied; the purification column placed back in the tube and the column was re-spin for 1 minute at maximum speed (14,000 rpm) as a dry run.

- The collection tube containing the flow-through solution was discarded and the column transferred to a sterile 1.5ml micro centrifuge tube.

- 100µl of Elution Buffer was added to the center of the column membrane to elute genomic DNA.

- The column was incubated for 2 minutes at room temperature and then centrifuged for 1 minute at 10,000 rpm.

- The purification column was discarded and the purified DNAwas poured in 1.5mL Eppendorf tubeand stored at -20^0C for PCR.

3.6.2 Detection of Brucella DNA by Conventional PCR

The genomic DNA of *Brucella* spp was detected using oligonuclides employing forward primer B4 (5′ TGG CTC GGT TGC CAA TAT CAA 3′) and reverse primer B5 (5′ CGC GCT TGC CTT TCA GGT CTG 3′) were used to amplify a 798bp fragment of the brucella DNA strands (Baily et al., 1992).

10µl of the mastermix containing 1 x buffer, 1.5µM $MgCl_2$, 200 µM of each dNTPs, 2U of Taq DNApolymerase was added to 0.2µl of 100 µM forward primer, 0.2µl of 100 µM reverse primer and 3.0µl of the purified target DNA extract to make a final volume of 20µl of the PCR reaction mixture. The PCR amplification was done in a thermal cycler (Bio-Rad, USA).

The primary PCR amplification involved an initial denaturation step at 94^0C for 5mins, followed by denaturation, annealing and extension for 35 cycles at 94^0C for 1 min, 56.5^0C for 1 min and 72^0C for 1 min, respectively and a final extension phase at 72^0C for 7 mins.

A reagent control containing all the PCR reagents without DNA was included to evaluate the success of the amplification and purity of the reagents.

Important parameters such asthe required annealing temperature, primer concentration, Mg2+ concentration, extension time and the amount and quality of Taq DNA polymerase were adjusted and several tests performed in order to obtain maximum band intensity for the Brucella gene amplicons and optimize PCR conditions.

Gel electrophoresis

The PCR amplification products were resolved in a 2 % (w/v) agarose gelcontaining 1xTBE buffer (100mM Tris-HCl (pH 8), 90 mM boric acid and 1 mM Na_2EDTA) and stained with ethidium bromide (0.5 μg/ml) and run at 75V for 45 mins.

A 1000 base pair molecular ladder (Promega, USA) was run concurrently.A visible band of 798 base pairs was considered as a positive reaction for *B. melitensis* and *B*. ovis. A positive control (based on DNA from *B. melitensis* and *B. ovis*) and a negative control (DNases and RNases free water) were included in all tests.The agarose gel was visualized with UV transilluminator for the presence of 798 bp bands. The photographs of the stained DNA in gel were taken using gel viewer (Gelmax 125 Imager, UVP, Cambridge, UK) for documentation and determination of the expected bands.

3.7 Questionnaire

Based on existing literature, a structured questionnaire was developed and administered to assess the pastoralists' practices to find out the risk factors which could contribute to or limit the spread of Brucellosis from one animal to another. The questionnaire contained questions covering flock dynamics, risk factors associated with brucellosis 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, who were mainly household heads, were interviewed orally in Swahili and some translated to Maasai and their responses entered into the open data kit (ODK).

3.8 Data analysis

The raw data from RBT, cELISA, molecular tests and ODK questionnaire were entered into a Microsoft excel spreadsheet which was used to compute descriptive statistics. The data was then entered in SPSS®Version 20.0 and Instat plus where logistic regression analysis was used to find possible risk factors that were associated with Brucellosis. Odds ratios were used to show the degree of association between the identified risk factors and brucellosis infection.

3.9 Ethical approval

The approval for conducting this project was sought from the International Ethical Research Committee from ILRI in Nairobi, which is recognized by NACOSTI, Kenya. Consent was obtained from all farmers that participated in this project.

CHAPTER FOUR: RESULTS

4.0 Introduction

This chapter will present the findings of this research. These findings are presented based on objectives that the study sought to achieve by determining the prevalence of ovine and caprine brucellosis and analyzing factors associated with animal infection in Kajiado County.

4.1 Sheep and goat flock demographics in Kajiado County

Data were obtained from a total of 130 flocks: 27 flocks from Ildamat ward and 25 flocks from Matapato South ward, in Kajiado central sub county, 31flocks from Kaputiei North ward and 21 flocks from Kenyawa Poka ward in Kajiado East sub county and 26 flocks from Iloodokilani ward in Kajado West sub county. A total of 1560 sheep and goats were sampled. Out of these, 887 were sheep and 673 were goats. All the 130 flocks had a total of 14,626 sheep and goats, of which 8,610 were sheep and 6,016 were goats. Serum samples were obtained from 145 goats and 179 sheep in Ildaamat ward, 145 goats and 167 sheep in Iloodokilani ward, 104 goats and 268 sheep in Kaputiei north ward, 120 goats and 132 sheep in Kenyawa poka and 159 goats and 141 sheep in Matapato south ward.

4.2 Estimated flock seroprevalence of brucellosis by RBT in Kajiado County

The overall flock seroprevalence of brucellosis in sheep and goats in Kajiado County based on RBT screening was 5.38% (7/130). The flock seroprevalence in sheep was 2.31% (3/130) and 3.85% (5/130) in goats. The highest flock seroprevalence of brucellosis in sheep and goats in Matapato south ward was 12% (3/25) followed by Kenyawa poka at 9.52% (2/21), Ildaamat at3.7% (1/27) and Kaputiei north at 5.77% (3/52). Iloodokilani ward had no positive serum samples based on RBT tests in sheep and goats. The flock seroprevalence in sheep was 8%

(2/25) in Matapato south, but there were no positive cases using RBT in sheep in Ildaamat,

Kenyawa poka, Kaputiei north and Iloodokilani wards.The flock seroprevalence in goats was 8%

(2/25) in Matapato south, 9.52% (2/21) in Kenyawa poka and 3.7% (1/27) in Ildaamat ward.

There were no positive cases by RBT in goats in Iloodokilani ward.

Table 1: Estimated flock seroprevalence of Brucellosis by RBT distributed by wards in Kajiado County

Administrative ward	RBT positive samples	Total samples	Brucella prevalence by RBT
Ildaamat	1	27	3.7%
Iloodokilani	0	26	0.0%
Kaputiei north	1	31	3.22%
Kenyawa poka	2	21	9.5%
Matapato south	3	25	12%

4.3 Detection of *Brucella melitensis* by cELISA

All the positive serum samples by RBT and others selected from the 130 flocks based on history

of abortion and from sick ewes and does were further tested using cELISA for *Brucella*

melitensis antibodies. A total of 320 serum samples were subjected to cELISA.Two serum

samples tested positive for *Brucella melitensis* by cELISA. One of these samples, collected from

a goat, had also tested positive by RBT. Both samples were from Matapato south administrative

ward.

Figure 2: Image of the ELISA plate showing Brucella melitensis positive sample in well F6.

4.4 Determination of flock molecular prevalence of Brucellosis by conventional PCR

Whole blood samples corresponding to the serum samples that had tested positive in RBT and cELISA together with other serum samples that were randomly selected from other flocks were subjected to conventional PCR. *B. melitensis* and *B. ovis* were confirmed by conventional PCR using specific primers for their IS711 and omp31 (JX081250.1) genes that gave the anticipated bands of 798 base pairs. Figure 3 below shows an image obtained from the GelMax imager.

1   2 3 4   5 6 7   8   9 Lad 10 11 12 13 14 15 16 17 18 19
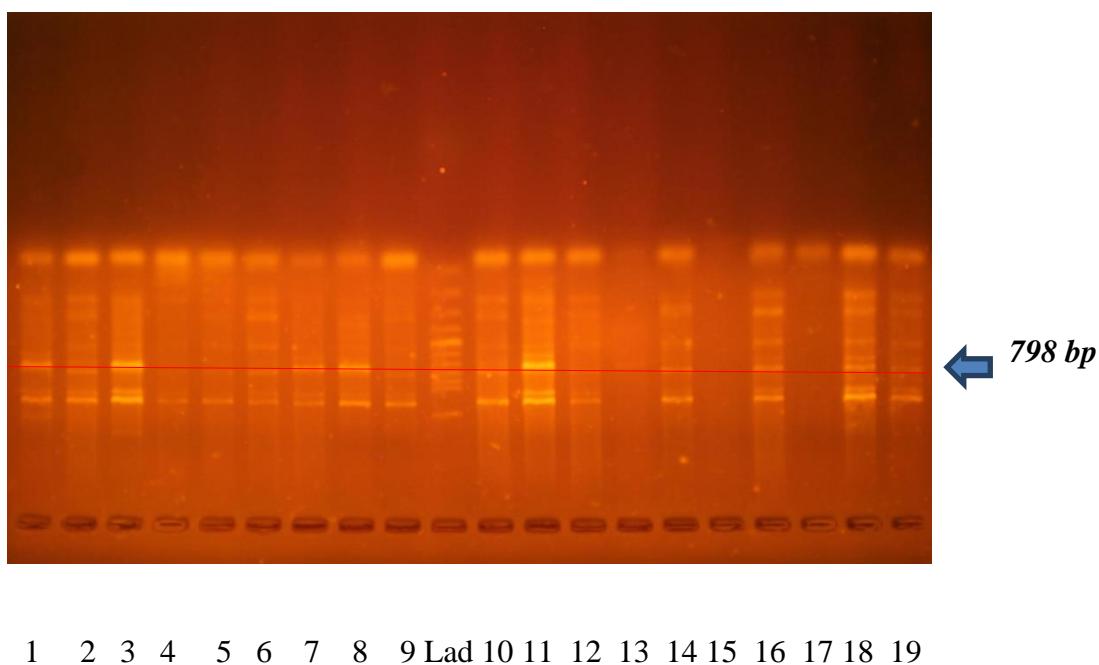
Figure 3: An image showing the bands obtained at 798 base pairs molecular weight on the Gelmax imager after electrophoresis.

The flock molecular prevalence of Brucellosis in sheep and goats was highest in Kenyawa poka ward at 35.7% (5/14) followed by Matapato south ward at 33.3% (4/12), Ildaamat ward at 18.2% (2/11) and Kaputiei north ward at 8.3% (1/12). Iloodokilani ward had no positive PCRtests in sheep and goats.

Table 2: Estimated flock molecular prevalence of Brucella by conventional PCR in the selected administrative wards

Administrative ward	PCR positive flocks	Total flocks	Brucella prevalence by PCR
Ildaamat	0	11	18.2%
Iloodokilani	-	-	-
Kaputiei north	1	12	8.3%
Kenyawa poka	5	14	35.7%
Matapato south	4	12	33.3%

4.5 Comparison of Brucella positivity between sheep and goats in the selected wards

Approximately1.5% (2/130) of serum samples from sheep and 3.8% (5/130) from goats tested positive for Brucellosis using RBT. The serum samples from sheep from all selected flocks were negative for Brucella by conventional PCR. However, goats from 35.3% (12/34) of all selected flocks that were subjected to conventional PCR tested positive for Brucella.Conversely, sheep serum from 2 flocks in Matapato south tested positive for Brucella using RBT. Sheep from the remaining 4 administrative wards all tested negative. However, goats from 2 flocks in Kenyawa poka, one flock in Ildaamat, one flock in Kaputiei north and one flock in Matapato south administrative wards tested positive for Brucella by RBT. Among the selected flocks subjected to PCR, goats from 5 flocksin Kenyawa poka, 4 flocks in Matapato south, 2 flocks in Ildaamat and one flock in Kaputiei north administrative wards tested positive by conventional PCR.

Table 3: Comparison of Brucella positivity between sheep and goats in the selected administrative wards

Administrative ward	RBT Positive flocks		PCR Positive flocks	
	Sheep	Goats	Sheep	Goats
Ildaamat	0/27	1/27	0/10	2/6
Iloodokilani	0/26	0/26	-	-
Kaputiei north	0/31	1/31	0/18	1/7
Kenyawa poka	0/21	2/21	0/8	5/13
Matapato south	2/25	1/25	0/9	4/8
% positivity	1.5%	3.8%	0.0%	35.3%

4.6 Predisposing factors for flock and human infection

The factors that predispose flocks and humans to Brucella infection were analysed using descriptive statistics and grouped frequency distribution for categorical variables and measures of dispersion and central tendency for continuous variables were computed. Several factors were found to be common practices among pastoralists and these have been associated with Brucellosis.

It was found that most flocks kept at least seven sheep in their flocks. However, several flocks did not keep goats. The average flock size of sheep in Kajiado County was 65 sheep per flock. The mean flock size of sheep was 63 in Ildaamat, 46 in Matapato south, 78 in Iloodokilani, 59 in Kenyawa poka and 77 in Kaputiei north ward. The average flock size of goats in Kajiado County was 47 goats per flock. The average flock size of sheep was 41 in Ildaamat, 53 in Matapato south, 43 in Iloodokilani, 61 in Kenyawa poka and 36 in Kaputiei north ward.

Table 4: Distribution of sheep flock size across the administrative wards in Kajiado County

Ward	Mean	Min	Max	Median	SD
Ildaamat	63.56	0	300	48	61.58
Matapato south	46.08	7	110	40	27.94
Iloodokilani	78.81	21	287	55	69.26
Kenyawa poka	59.81	22	120	58	27.69
Kaputiei north	77.16	12	230	70	49.99

Table 5: Distribution of goat flock size across the administrative wards in Kajiado County

Ward	Mean	Min	Max	Median	SD
Ildaamat	41.89	0	200	30	41.21
Matapato south	53.68	16	241	36	49.06
Iloodokilani	43	0	150	32.5	35.52
Kenyawa poka	61.38	0	200	42	49.29
Kaputiei north	36.65	0	170	25	45.08

History of abortion in ewes and does in the flock within the past 12 months was found to be statistically significant. Approximately 56.92% (74/130) of the flocks in Kajiado County had experienced cases of abortion in their flocks within the past 12 months. 80% (104/130) flocks were using communal grazing pasture lands for their livestock. The animals were allowed to mix freely with animals from other flocks during grazing period. About 96.92% (126/130) of flocks In Kajiado County shared common watering points with other flocks. These included water pans, rivers, boreholes and wells where animals from different flocks could mix freely to drink water. In addition, 50.77% (66/130) of flocks had new entries of animals from other regions added in the past 12 months. These were either bought from livestock auction markets, other pastoralists or given as gifts from friends or payment for dowry or other debts. Furthermore, 88.46% (115/130) of flocks were moved to other regions in search of pastures and water during the dry

seasons. These were either short distance movements to the neighbouring regions where the pastoralists would return the animals back home in the evening or long distance movements where the herdsmen would go as far as Narok County or crossing the border to Tanzania and stayed there until the dry season passed. This was a common practice during droughts years or months. About42.31% (55/13) of flocks shared breeding bucks and rams with neighbouring flocks to serve their ewes and does during the breeding seasons. They either borrowed the breeding males or hired them at an agreed fee and these males would stay in the flock for an agreed period of time during the breeding season. Other pastoralists would agree with the owner of the breeding males to allow the two flocks to graze together during the breeding season.

A relatively large proportion of the pastoralists interviewed in the 130 flocks lacked knowledge of ovine and caprine brucellosis. Pastoralists from only 30% (39/130) of the flocks were aware that Brucella exists in sheep and goats. A significantly high proportion of pastoralists also did not seek veterinary services whenever their sheep or goats were sick. Only pastoralists from 40% (52/130) of the flocks sought veterinary services when their animals were diseased. The other 60% of the pastoralists either left the animal to recover naturally or attended to the sick animals by themselves mostly using traditional herbs to treat the diseases. Five factors were analysed and all were found to be common practices among pastoralists hence can be risks for transmission of Brucella from flocks to humans. Almost 90.77% (118/130) of the pastoralists interviewed never used protective gear such as gloves, gum boots and overalls when handling aborted materials or when assisting the animals during delivery. 3.85% (5/130) always wore protective gear while 5.38% (7/130) wore them occasionally. Similarly, 93.08% (121/130) of the pastoralists never consumed raw milk. They always boiled milk before consumption. However, 0.77% (1/30) of the pastoralists often consumed raw milk while 6.15% (8/130) occasionally consumed raw milk,

34

but 79.23% (103/130) of pastoralists reported that they have never consumed raw meat.

However, 0.77% (1/30) of the pastoralists always consumed raw milk while 20% (26/130), while

occasionally consuming raw meat and 50% (65/130) of the pastoralists would consume raw

blood. 48.46% (63/130) reported to occasionally consumed raw blood while 1.54% (2/130)

always consumed raw blood. Finally, about 71.54% (93/130) of the pastoralists did not have

knowledge of zoonotic diseases while 28.46% (37/130) were aware that animal diseases can

infect humans.

Table 6: Descriptive analysis of the predisposing factors for animal and human infection with

Brucella in Kajiado County

Predisposing factor to Brucellosis	Nature of response	Number of flocks out of 130	Percentage (%)
History of abortion in the flock	Yes	74	56.9
Common grazing pastures	Yes	104	80
Common watering points	Yes	126	96.9
New animal entries into flock	Yes	66	50.7
Animal movement in dry season	Yes	115	88.5
Sharing of breeding males	Yes	55	42.3
Knowledge of brucellosis	No	93	71.5
Seeking veterinary services	No	78	60
Wearing protective gear when handling aborted materials or neonates	Never	118	90.7
	occasionally	7	5.4
	Always	5	3.9
Consuming raw meat	Never	121	93
	Occasionally	8	6.2
	Always	1	0.8
Consuming raw blood from animals	Never	103	79.2
	Occasionally	26	20
	Always	1	0.8
New animal entries into flock	Never	65	50
	Occasionally	63	48.5
	Always	2	1.5

4.7 Analysis of the risk factors associated with Brucellosis in sheep and goats

The risk and protective factors associated with flock infection were analysed by logistic regression. Eight factors were examined at the univariate analysis of which six variables (*introduction of new animals in the flock, experiencing abortion in sheep and goat flocks, contact with other flocks in common grazing fields and watering points, sharing male animals with other flocks for breeding purposes, knowledge of brucellosis and seeking veterinary services*) were significantly associated with brucella flock positivity.

The following factors were found to be independently associated with flock brucellosis test positivity in sheep and goats: *experiencing abortion in sheep and goats flock (odds ratio (OR) = 3.09; 95% CI), introduction of new animals into the flock (OR = 1.33; 95% CI), mixing with other flocks in communal grazing fields and common watering points (OR = 1.56; 95% CI), sharing male animals with other flocks for breeding purposes (OR = 1.03; 95% CI), knowledge of brucellosis and other zoonotic diseases (OR = 0.85; 95% CI) and seeking of veterinary services (OR = 0.23; 95% CI).*

4.7.1 Univariate analysis of the predisposing factors for brucellosis

Univariate analysis showed history of abortion, mixing of flocks in grazing fields and common watering points, introduction of new livestock to the flock, sharing male animals with other flocks for breeding purposes, knowledge of brucellosis, seeking veterinary services to be associated with the test positivity for Brucella infections.

Flocks that had history of abortion were 3.09 time more likely to have at least one sheep or goat test positive for brucella relative to those flocks that had not experienced any case of abortion before (OR=3.09). Similarly, flocks that were mixing freely with other flocks at communal grazing fields and watering points were 1.56 times more likely to have an animal test positive for

brucella (OR=1.56) relative to animals that were restricted to grazing in private land, provided with feeds like hay at home and had private water sources. Flocks that regularly introduced new animals into their flocks were 1.33 times more likely to have at least one sheep or goat test positive for brucella relative to flocks that were not (OR=1.33). Flocks that were sharing male animals with other flocks for breeding purposes were 1.03 times more likely to have brucellosis relative to flocks strictly using their own males for breeding (OR=1.03).

Two factors were negatively associated with brucellosis positive test. These included farms that had knowledge of brucellosis and other zoonotic diseases (OR=0.85) and those who often sought veterinary services (OR=0.23).

4.7.2 Multivariable analysis of risk factors for exposure to brucellosis

Out of the six flock level risk factors that were significantly associated with infection in univariate analysis, four remained significant in the multivariable logistic regression model. The factor "sharing males for breeding purposes" lost its significance in determining the brucella flock test positivity. This shows that its association was confounded by another factor in the model, probably "mixing of flocks in common grazing and watering points'. The factor "seeking veterinary services" also lost significance implying that its association with brucellosis positivity might have been confounded by another factor, most likely "knowledge of brucella and other zoonotic diseases". The odds ratios (ORs) for the other factors retained their association and showed no much difference from the univariable analysis.

Table 7: Multivariate regression analysis of risk factors for flock Brucella infection

Variable	Estimate	Odds ratio (OR)	Confidence intervals		p-value
			Lower	Upper	
History of abortion.	0.697	2.64	0.097	1.488	0.0164
Mixing of flocks (grazing & watering)	0.853	2.67	0.083	2.346	0.0337
Entry of new animals into the flock.	0.682	1.25	0.210	3.039	0.0742
Knowledge on Brucella.	0.165	0.848	0.322	4.314	0.0803

CHAPTER 5: DISCUSSION

This study intended to detect Brucella by combining serological tests with molecular tests and analyze risk factors for animal infection in sheep and goats in Kajiado County. Three tests were used for accurate detection of brucella, which is akin to eradication or elimination of brucellosis (Tsevelmaa *et al*., 2018). Samples of serum and whole blood were randomly collected from 1560 sheep and goats in Kajiado County and screened using RBT. 320 serum samples, were selected and further tested using cELISA for *Brucella melitensis* and *Brucella ovis* antibodies. Whole blood samples corresponding to the serum samples that tested positive in both serological tests and other randomly selected negative samples, making a total of 72 samples, were further subjected to conventional PCR.

The overall flock prevalence for brucellosis was 11.54% (2.31% in sheep and 10.00% in goats). This morbidity at flock level implies that brucellosis has significant economic implications and should be viewed as a public health concern. This is because Brucella pathogens can rapidly spread from a single animal to all animals in the flock and beyond (González-Espinoza et al., 2021). The overall individual animal level prevalence of brucellosis was found to be 0.96% (0.34% in sheep and 1.78% in goats). This is a lower estimate when compared to the seroprevalence reported in the country by Nakeel *et al*., 2016 in Kajiado (8.6% in sheep and 7.3 in goats), Kahariri, 2018 in Marsabit (16.09% in goats and 11.89% in sheep), Obonyo, 2018 in Garissa (24.3% in goats and 12.5% in sheep). This could be attributed to the differences in the management practices in these areas and the level of knowledge of the pastoralists about brucellosis over time since the disease was confirmed to be present in the country.

The molecular flock prevalence of brucella obtained by PCR in this study (21.43%) is higher than the flock sero-prevalence obtained by RBT and cELISA (6.92%). These findings are in

agreement with previous studies (Mohseni *et al*., 2017). The stage of infection and the higher sensitivity of PCR may explain why PCR assay had better outcome compared to the serological methods in diagnosis of the disease. Moreover, PCR assay can detect small traces of DNA in samples, while serological techniques only detect antibodies formed by the host against the Brucella pathogens (Yasmin *et al*., 2018).

There was a significant difference in the prevalence of Brucella infection between sheep and goats in Kajiado coutny. The higher prevalence of the disease in goats than in sheep was comparable with other studies (Osoro et al., 2015, Kelkay et al., 2018). This species prevalence difference might be because sheep are more resistant to infection compared to goats and flocks with high numbers of livestock would have a low prevalence of the disease. Moreover, goats are the primary hosts of *B. melitensis*, and sheep are not significantly infected even when kept together with goats. In addition, infection in goats can develop to chronic for a long time while sheep have been reported to be resistant to re-infection (Costa *et al*., 2016).

In analysing the risk factors associated with flock level Brucella infection it was found that the presence of female animals that had aborted in the flock was significantly associated with flock brucellosis positivity. Abortion in livestock represents the major complaint attributed to Brucella infections. Females infected with *Brucella spp*s are known to shed highly concentrated volumes of Brucella organisms in milk, placental membranes and aborted foetuses and they may continue shedding the organisms for several months resulting into environmental contamination which spreads these organisms among animals of the same flock and other flocks during free mixing in grazing and watering places (Kelkay *et al*., 2017).

The introduction of new animals to the sheep and goat flocks from unscreened flocks was significantly associated with Brucella flock test positivity in this study (OR=1.33; 95% CI). This

was in agreement with several other studies which noted that introduction of animals from flocks whose brucellosis status was unknown was significantly associated with brucellosis infection in sheep and goat flocks. Other studies suggest that the introduction of infected animals can lead to an increase in the individual level prevalence due to the fact that the longer the animals stay in the flock and they are in contact with the rest of the flock, the higher is the risk of spread of brucellosis (Asiimwe *et al*., 2015).

Mixing of animals amongst different flocks in communal grazing lands and common watering points have also been found be associated with flock brucella test positivity and this may potentiate the transmission of brucellosis between flocks (Tegegn, 2016). Evidence suggests that failure of most brucellosis control strategies could be explained by uncontrolled movement of animals and lack of efficient measures to prevent entry of infected animals, and this is very common in pastoral communities. Proper screening of new livestock is perhaps one of the effective control strategies for ovine and caprine brucellosis (Boukary, 2013).

Pastoralist knowledge about brucellosis and seeking veterinary services were also factors that were significantly associated with brucellosis flock test positivity as protective factors. This helps pastoralists to take precautionary measures in preventing transmission of the disease among flocks (Demena, 2019)

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

CONCLUSION

A combination of serological and molecular tests provided a relatively faster and more accurate diagnosis of *Brucella* and its detection in sheep and goat flocks in Kajiado County. The findings of this research have shown the significance of ovine and caprine brucellosis in Kajiado County and presented the associated risk factors in animals and their possible zoonotic implications.

A significant proportion of sheep and goats kept in Kajiado County are infected with *B. melitensis* and *B. ovis*. Since a large proportion of infections in people are caused by *B. melitensis*, the most common *Brucella spp* of small ruminants, there is a high risk for public health. Such findings of a significant level of infection in the absence of any prevention and control strategy in form of vaccination against the disease indicate the occurrence of natural infection.

Risk factor analysis concluded that experiencing abortion in sheep and goats flocks, introduction of new animals into the flock, mixing with other flocks in communal grazing fields and common watering points, sharing male animals with other flocks for breeding purposes, knowledge of brucellosis and other zoonotic diseases and seeking veterinary services were significantly associated with brucellosis infection in sheep and goat flocks in the pastoral regions of Kajiado county. There is close contact among the pastoral community and livestock, making the presence of the disease in small ruminants an indicator of potential risk for the pastoralists. Moreover, these pastoral areas constitute an important source of small ruminants in the Kenyan and international markets and can contribute to the spread of the infection to other areas. Prevention and control of the disease in livestock in this region will definitely result in reduced incidence of the brucellosis in humans.

RECOMMENDATIONS

- Assessment of the zoonotic effects of brucellosis on humans, is worthy as the prevalence is significant.

- One health approach and combined efforts among the veterinary and public health professionals are very important in controlling brucellosis in livestock, humans and wildlife.

- Create awareness in the county on routine flock management, disease management and risk of brucellosis.

- Further research on the molecular detection of *Brucella* species in humans and understanding the linkage between human and animal infection in the region is imperative.

REFERENCES

Akoko J. M., Pelle R., Kivali V., Schelling E., Shirima G., Machuka E. M., Mathew C., Fèvre E. M., Kyallo V., Falzon L. C., Lukambagire A. S., Halliday J. E. B., Bonfoh B., Kazwala R., & Ouma, C. (2020). Serological and molecular evidence of Brucella species in the rapidly growing pig sector in Kenya. *BMC Veterinary Research*, *16*(1), 133. https://doi.org/10.1186/s12917-020-02346-y

Akoko J. M., Roger P., AbdulHamid S.L., Eunice M. M. , Nthiwa D, Coletha M., Eric M. F., Bernard B., Elizabeth A. J. Cook., Othero D. , Bassirou B. , Rudovick R. K., Gabriel S., Schelling E., Jo E. B. Halliday and Ouma C. (2021);Molecular epidemiology of Brucella species in mixed livestock-human ecosystems in Kenya.Scientifc Reports. 11:8881https://doi.org/10.1038/s41598-021-88327-z

Alemneh, T., & Akeberegn, D. (2018). *A Review on Small Ruminants Brucellosis*. 16.

Al Dahouk, S. Evaluation of genus-specifc and species-specifc real-time PCR assays for the identifcation of Brucella spp. Clin. Chem. Lab. Med. 45, 1464–1470 (2007).

Amjadi, O.; Rafiei, A.; Mardani, M.; Zafari, P.; Zarifian, A. (**2019):** A review of the immunopathogenesis of Brucellosis. Infect. Dis., 51, 321–333.

Alton GG, Jones LM, Angus RD, Verger JM (1998): Techniques for the Brucellosis Laboratory. Insititute National de la Recherche Agronomique, Paris, France.

APHA Scientific. (2014).COMPELISA 160 & 400 a competitive ELISA kit for the detection of antibodies against Brucella in serum samples Instructions for use (for in-vitro and animal use only); p. 1–4.

Assenga JA, Matemba LE, Muller SK, Malakalinga JJ, Kazwala RR. (2014). Epidemiology of Brucella infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. BMC Vet Res.11:189.

Asiimwe, B. B., Kansiime, C., & Rwego, I. B. (2015). Risk factors for human brucellosis in agro-pastoralist communities of south western Uganda: A case–control study. *BMC Research Notes*, *8*(1), 405. https://doi.org/10.1186/s13104-015-1361-z

Baily GG, Krahn JB, Drasar BS, Stoker NG (1992); Detection of Brucella melitensis and Brucella abortus by DNA amplification.The Journal of Tropical Medicine and Hygiene, 95(4):271-275 PMID: 1495123

Bang B. (1897); The etiology of epizootic abortion. J Comp Pathol Ther.10(125):IN2–149.

Berger S. (2016);Brucellosis: Global Status. Los Angeles, CA: GIDEON Informatics, Inc.

Blair Hedges S, Kumar S: (2003). Genomic clocks and evolutionary timescales. **Trends Genet.**19(4),200–206.

Blasco J M and Molina-Flores B. (2011). Control and eradication of *Brucella melitensis* infection in sheep and goats. *Veterinary Clinics of North America (Food Animal Practice)* 27 (1): 95–104.

Bodenham, R. F. (2020): Prevalence and speciation of brucellosis in febrile patients from a pastoralist community of Tanzania. Sci. Rep. [Internet] https://doi.org/10.1038/s41598-020-62849-4.

Boukary, A.R., (2013): Sero-prevalence and potential risk factors for Brucella spp. Infection in traditional cattle, sheep and goats reared in urban, periurban and rural areas of niger. Plos one. **8**(12): p. e83175.

Buzgan T, Karahocagil MK, Irmak H, (2010); Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. Int J Infect Dis.14(6):e469–e478.

Charyapkhan C, Akhmetzhan AS, Nikolav PI, Sholpan AB, (2019): Economic and health burden of Brucellosis in Kazakhstan. Wiley online library, zoonoses and public health 66 (5), 487-49.

Corbel M (2006); Brucellosis in Humans and Animals: FAO, OIE, WHO. Available: http://www.who.int/csr/resources/publications/Brucellosis.pdf.

Costa L.F., Moisés S.P., Laís B.G.,Karoene A.S., Rodrigo P.M., Juliana P.S., Luize N.G., Almeida A.C., Aurora M.G., Marcos X.S., Tatiane A.P. and Renato L.S.,(2016): Serologic and molecular evidence of *Brucella ovis* infection in ovine and caprine flocks in the State of Minas Gerais, Brazil. BMC Res Notes 9:190; DOI 10.1186/s13104-016-1998-2.

Dean AS, Crump L, Greter H, Schelling E, Zinsstag J. (2012); Global burden of human brucellosis: a systematic review of disease frequency. PLoS Negl Trop Dis.6:e1865.

Demena, G. K. (2019). Small ruminant brucellosis and awareness of pastoralist community about zoonotic importance of the disease in yabello districts of Borena zone Oromiya regional state, Southeren Ethiopia. *International Journal of Infectious Diseases*, *79*, 72. https://doi.org/10.1016/j.ijid.2018.11.183

Desta AH, (2016): One Health: An Integrated Approach for Disease Prevention and Control in Pastoral Areas of Ethiopia. *Journal of Health, Medicine and Nursing.* 22: 2006–2011.

Dohoo, I. R., Martin, S. W. and Stryhn, H. Methods in Epidemiologic Research (VER Inc., 2014).

Eisenberg T, Hamann HP, Kaim U, (2012); Isolation of potentially novel Brucella spp. from
frogs. Appl Environ Microbiol. 78(10):1–12. PubMed PMID: 22407680; PubMed Central
PMCID: PMCPMC3346351.

FAO. (2017). *Africa Sustainable Livestock 2050. Country Brief Kenya*.

Franc K. A., Krecek, R. C., Häsler, B. N., & Arenas-Gamboa, A. M. (2018). Brucellosis
remains a neglected disease in the developing world: A call for interdisciplinary action.
BMC Public Health, *18*(1), 125. https://doi.org/10.1186/s12889-017-5016-y

Godfroid J, Al Dahouk S, Pappas G, (2013). A "One Health" surveillance and control of
brucellosis in developing countries: moving away from improvisation. Comp Immunol
Microbiol Infect Dis.36(3):241–248. PubMed PMID: 23044181.

González-Espinoza, G.; Arce-Gorvel, V.; Mémet, S.; Gorvel, J.-P. Brucella: Reservoirs and
Niches in Animals and Humans. Pathogens 2021, 10, 186. https://doi.org/10.3390/
pathogens10020186

**Gutiérrez-Jiménez, C.; Mora-Cartín, R.; Altamirano-Silva, P.; Chacón-Díaz, C.; Chaves-
Olarte, E.; Moreno, E.; Barquero-Calvo, E. (2019);** Neutrophils as Trojan Horse
Vehicles for Brucella abortus Macrophage Infection. Front. Immunol. 10, 1012.

**Karim, M.F.; Maruf, A.A.; Yeasmin, F.; Shafy, N.M.; Khan, A.H.N.A.; Rahman,
A.K.M.A.; Bhuiyan, M.J.S.; Hasan, M.M.; Karim, M.R.; Hasan, M.T. (2019)**;.
Histopathological changes of brucellosis in experimentally infected guinea pig.
Bangladesh J. Vet. Med., 17.

Kelkay, M. Z., Gugsa, G., Hagos, Y., & Taddelle, H. (2017). Sero-prevalence and associated
risk factors for Brucella sero-positivity among small ruminants in Tselemti districts,

Northern Ethiopia. *Journal of Veterinary Medicine and Animal Health*, *9*(11), 320–326.

https://doi.org/10.5897/JVMAH2017.0604

KNBS; (2019). Kenya national population and hosing census.

Kunda J, Fitzpatrick J, French N, Kazwala R, Kambarage D,.(2010); Quantifying risk

factors for human brucellosis in rural northern Tanzania. PLoS One. 5:e9968.

Letesson, J..; Barbier, T.; Zúñiga-Ripa, A.; Godfroid, J.; De Bolle, X.; Moriyón, I. (2017);

Brucella Genital Tropism: What's on the Menu. Front. Microbiol, 8.

Luce R, Snow J, Gross D, (2012); Brucellosis seroprevalence among workers in at-risk

professions: northwestern wyoming, 2005 to 2006. J Occup Environ Med.54(12):1557–

1560.

Makita K, Fevre EM, Waiswa C, Kaboyo W, Eisler MC,. (2011): Spatial epidemiology of

hospital-diagnosed brucellosis in Kampala, Uganda. Int J Health Geogr. 10:52.

Makita K, Fèvre EM, Waiswa C, (2010): How human brucellosis incidence in urban Kampala

can be reduced most efficiently? A stochastic risk assessment of informally-marketed

milk. PloS One. 5(12):e14188.

Mandal SS, Duncombe L, Ganesh NV, Sarkar S, Howells L, Hogarth PJ, Bundle DR(2017),

McGiven J. Novel solutions for vaccines and diagnostics to combat brucellosis. ACS

Cent Sci.;3(3):224–31.

Meng F, Xiangpo Pan,Wenzhen Tong (2018); Rifampicin versus streptomycin for brucellosis

treatment in humans: A meta-analysis of randomized controlled trials. Tropical

agriculture. https://doi.org/10.1371/journal.pone.0191993

Meyer K. F, Shaw E. B. (1920): Comparison of the morphologic, Cultural and Biochemical

Characteristics of B. Nov Gen I J Infect Dis. 27(3):173–184.

Migisha, R. (2018): Prevalence and risk factors of brucellosis among febrile patients attending a

community hospital in south western Uganda. Sci. Rep. 8, 15465.

Mcdermott, J. J., Grace, D., & Zinsstag, J. (2013): Economics of brucellosis impact and

control in low-income countries: -EN- -FR- -ES-. *Revue Scientifique et Technique de

l'OIE*, *32*(1), 249–261. https://doi.org/10.20506/rst.32.1.2197

Mohseni, K., Mirnejad, R., Piranfar, V., & Mirkalantari, S. (2017): A Comparative

Evaluation of ELISA, PCR, and Serum Agglutination Tests For Diagnosis of Brucella

Using Human Serum. *Iranian Journal of Pathology*, *12*(4), 371–376.

Moreno E. (2014): Retrospective and prospective perspectives on zoonotic brucellosis. Front

Microbiol. 5:213. PubMed PMID: 24860561; PubMed Central PMCID: PMC4026726.

Mustafa A, Nicoletti P, editors. FAO, WHO, OIE, (1995): guidelines for a regional

brucellosis control programme for the Middle East. Workshop of

Amman, Jordan, Ammended at the Round-Table.

**Njeru, J., Wareth, G., Melzer, F., Henning, K., Pletz, M. W., Heller, R., & Neubauer, H.

(2016):** Systematic review of brucellosis in Kenya: Disease frequency in humans and

animals and risk factors for human infection. *BMC Public Health*, *16*(1), 853.

https://doi.org/10.1186/s12889-016-3532-9

Nyariki, D. M., and Amwata, D. A. (2019): The value of pastoralism in Kenya: Application of

total economic value approach. *Pastoralism*, *9*(1), 9. https://doi.org/10.1186/s13570-019-

0144-x

Obonyo M.O (2018); sero-prevalence and factors associated with brucellosis in goats and sheep and assessment of pastoralists, knowledge attitude and practices towards brucellosis in garissa county**. http://hdl.handle.net/123456789/4634

Olsen SC, Palmer MV (2014): Advancement of knowledge of Brucella over the past 50 years. Vet Pathol.;51(6):1076–89.**Onono J, Mutua P, Kitala P and Gathura P (2019):** Knowledge of pastoralists on livestock diseases and exposure assessment to brucellosis within rural and peri-urban areas in Kajiado, Kenya. F1000Research [version 1; peer review: 1 approved],:1916

Onono J, Mutua P, Kitala P and Gathura P (2019). How to cite this article: Knowledge of pastoralists on livestock diseases and exposure assessment to brucellosis within rural and peri-urban areas in Kajiado, Kenya. F1000Research [version 1; peer review: 1 approved],:1916

Osoro, E. M., Munyua, P., Omulo, S., Ogola, E., Ade, F., Mbatha, P., Mbabu, M., Ng'ang'a, Z., Kairu, S., Maritim, M., Thumbi, S. M., Bitek, A., Gaichugi, S., Rubin, C., Njenga, K., & Guerra, M. (2015): Strong Association Between Human and Animal Brucella Seropositivity in a Linked Study in Kenya, 2012–2013. *The American Journal of Tropical Medicine and Hygiene*, *93*(2), 224–231. https://doi.org/10.4269/ajtmh.15-0113

Pedersen K, Quance CR, Robbe-Austerman S, Piaggio AJ, Bevins SN, Goldstein SM, Gaston WD, DeLiberto TJ. (2014): Identification of Brucella suis from feral swine in selected states in the USA. J Wildl Dis;50 (2):171–9.

Poester FP, Samartino LE, Santos RL (2013): Pathogenesis and pathobiology of brucellosis in livestock. Rev Sci Tech;32(1):105–15.

Ruiz-Ranwez, V.; Posadas, D.M.; Estein, S.M.; Abdian, P.L.; Martin, F.A.; Zorreguieta, A (2013): The BtaF Trimeric Autotransporter of Brucella suis Is Involved in Attachment to Various Surfaces, Resistance to Serum and Virulence. Plos one, 8, e79770.

Rodríguez, M.C., Viadas, C., Seoane, A., Sangari, F.J., López-Goñi, I., García-Lobo, J.M. (2012): Evaluation of the Effects of Erythritol on Gene Expression in Brucella abortus. PLoS ONE, 7, e50876.

Roth F, Zinsstag J, Orkhon D, (2003): Human health benefits from livestock vaccination for brucellosis: case study. Bull World Health Organ. 81(12):867–876.

Sarker M. A. S., Begum M. M., Shafy N. M., Islam M. T., Ehsan M. A., Bhattacharjee P. K., Rahman M. F., Melzer F., Neubauer H. and Rahman M. S. (2016): Seroprevalence and molecular diagnosis of brucella abortus and brucella melitensis in bangladesh. bangl. j. vet. med. 14 (2): 221–226 ISSN: 1729-7893 (Print), 2308-0922.

Saxena H. (2018): Brucellosis-in-Sheep-and-Goats-and-its-Serodiagnosis-and-Epidemiology.*Pdf* Retrieved June 29, 2020, from https://www.researchgate.net.

Singh, B. B., Dhand, N. K., & Gill, J. P. S. (2015): Economic losses occurring due to brucellosis in Indian livestock populations. *Preventive Veterinary Medicine*, *119*(3), 211–215. https://doi.org/10.1016/j.

Shirima GM, Kunda JS. (2016): Prevalence of brucellosis in the human, livestock and wildlife interface areas of Serengeti National Park, Tanzania. Onderstepoort J Vet Res.;83(1):2–5 Available from: http://ojvr.org/index.php/ojvr/article/view/1032.

Solís García, Solera J, Del Pozo J. (2017): Treatment of pulmonary brucellosis: a systematic review. Expert Rev Anti Infect Ther.15(1):33–42.

Stevens MG, Hennager SG, Olsen SC, (1994): Serologic responses in diagnostic tests for brucellosis in cattle vaccinated with Brucella abortus 19 or RB51. J Clin Microbiol. 32(4):1065–1066.

Swai ES, Schoonman L. (2009): Human brucellosis: seroprevalence and risk factors related to high risk occupational groups in Tanga Municipality, Tanzania. Zoonoses Public Health. 56(4):183–187.

Tegegn, A. H., & Feleke, A. (2016): Small Ruminant Brucellosis and Public Health Awareness in Two Districts of Afar Region, Ethiopia. *Journal of Veterinary Science & Technology*, *7*(4). https://doi.org/10.4172/2157-7579.1000335

Tewodros A. and Dawit A. (2015): Sero-Prevalence of Small Ruminant Brucellosis in and around Kombolcha, Amhara Regional State, North-Eastern Ethiopia. *Journal of Veterinary Science & Medical Diagnosis*, *04*(05). https://doi.org/10.4172/2325-9590.1000173

Weinstein RA, Singh K. (2009): Laboratory-acquired infections. Clin Infect Dis. 49(1):142–147.

Wyatt HV (2005): How Themistocles Zammit found Malta fever (brucellosis) to be transmitted by the milk of goats. **J. R. Soc. Med.** 98(10),451–454.

Yasmin E.T., Al Ghalya A.T., Waleed A.M., Osman M., Maryne J., Yannick C., Hadi A.L., Shekar B., Abeer A.H., Kaadhia A.K., Nasseb A.S., Rudaina A.B. and Eugene H. J., (2018): Serological, cultural and molecular evidence of Brucella melitensis infection in goats in Al Jabal Al Akhdar, Sultanate of Oman.John Wiley & Sons Ltd Veterinary Medicine and Science, 4, pp. 190–205

Von Bargen, K.; Gagnaire, A.; Arce-Gorvel, V.; de Bovis, B.; Baudimont, F.; Chasson, L.; Bosilkovski, M.; Papadopoulos, A.; Martirosyan, A.; Henri, S. (2015): . Cervical Lymph Nodes as a Selective Niche for Brucella during Oral Infections. PLoS ONE, 10, e0121790.

Zakaria, A. M. (2018): Comparative assessment of sensitivity and specifcity of rose bengal test and modifed in-house ELISA by using IS711 TaqMan Real Time PCR assay as a gold standard for the diagnosis of bovine brucellosis. Biomed. Pharmacol. J. 11(2), 951–957.

Zinsstag J, Schelling E, Waltner-Toews D, (2011): l. From "one medicine" to "one health" and systemic approaches to health and well-being. Prev Vet Med.101(3–4):148–156.

APPENDICES

Appendix 1: Certificate of Consent

I am willing to take part in this study on Brucellosis in sheep and goats in Kajiado County. I consent willingly on the following to be done (tick as appropriate):

An interview about practices in the small ruminant flock

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   Yes

flock.   No

Name of respondent_____

Signature of respondent _____

Date _____

If illiterate I witness that the consent form has been read and correctly interpreted to the respondent and they have participated in this research freely.

Name of witness ………………….. …………………..

Signature ………………………

Date……………………………

Name of researcher……………………………… Signature ……………………

Date ……………………………..

Appendix 2: Distribution of the flocks and animals sampled in the administrative wards of Kajiado County.

Ward	Flocks sampled	Species and number sampled		Total sheep and goats
Ildamat	**27**	Goat	145	**324**
		Sheep	179	
Iloodokilani	**26**	Goat	145	**312**
		Sheep	167	
Kaputiei North	**31**	Goat	104	**372**
		Sheep	268	
Kenyawa poka	**21**	Goat	120	**252**
		Sheep	132	
Matapato south	**25**	Goat	159	**300**
		Sheep	141	
				1560

Appendix 3: Questionnaire

Instruction to enumerator before starting the questionnaire

- *Provide a general introduction of the enumerator, the project and its goals and overall objectives of the survey*

- *Explain conditions of the interview and sampling of animals*

- *Obtain consent form signed*

The involvement of the rest of the family members, especially wife(s), will be encouraged as they can provide additional information about the shoats' management.

Identification questions – *to be completed by the enumerator alone before or after the interview*

1.   **Flock identification number**(Flock ID)

2.   **Date of interview (DD/MM/YYY)**

3.   **GPS codes** (automatically recorded with the tablet)

Latitude	
Longitude	

4. **Sub-county and ward** *(Single-answer question)

Sub-county	Ward	(Tick appropriately)
Kajiado East	Kaputiei North	
	Kenyawa Poka	
Kajiado Central	Ildaamat	
	Matapato South	
Kajiado West	Iloodokilani	

5. **How many sheep do you have now in <u>all </u>your flocks?**

Total sheep	

6. **How many goats do you have now in <u>all </u>your flocks?**

Total Goats	

7. **Regarding your ram(s) and buck(s) how did you get them?** (Single-answer question)

Raised my own	
Bought	
Rented	
Borrowed	

8.   **For how long do you keep them?** Expressed in years

	Age (years)

9. **How many breeding seasons do you have during the year for your <u>sheep and goats?</u>(breeding season: period when lambs and kids are born)** (Single-answer question)

One	

Two	
During the whole year	

10. **Do you know a disease called Brucellosis?**

Yes	
No	

11. **How many abortions did you notice during the last 12 months?** *This is referred as*

"early births" to be different from those born dead.

	Number
Sheep	
Goats	

12. **How many difficult births (dystocia) did you notice during the last 12 months?**

	Number
Ewes	
Does	

13. **How many lambs and kids died (up to 6 months old) during the last 12 months?**

	Number
Lambs	
Kids	

14. **Who own the land you use for grazing your sheep and goats?**(single answer question)

[The enumerator have to be sure that they are referring to sheep and goats]

Owned	
Public land	
Rented-in	
Other, specify	

15. **What is the main source of water do you usually use for watering your sheep and**

goats? (Single-answer question per column)

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

Thank You.

Blood sample collection

During the blood sampling procedure the body condition of the sampled animal will be recorded

*16. Body condition of sampled animals**A** full description of each code can be found at the*

protocol.

Score	SHEEP	GOATS

	1	2	3	4	5	6	1	2	3	4	5	6
1(emanciated)												
2 (thin)												
3 (normal)												
4 (slightly fat)												
5 (obese)												

*17. **Additional comments**. Any additional comment of interest will be included here by the*

enumerator.

Appendix 4: Work plan

Activity	Aug 2019	Sep 2019	Oct 2019	Nov 2019	Dec 2019	Jan 2020	Feb 2020	Mar 2020	Apr 2020	May 2020	Sep 2020
Proposal drafting	■										
Proposal presentation		■									
Proposal submission			■								
Field work				■							
Lab analysis					■						
Thesis preparation					■	■					
Departmental presentation of results						■					
Submission of thesis for examination							■	■	■		
Thesis defence										■	
Graduate											■

Appendix 5: Budget

This was part of the budget for the bigger research project.

BUDGET ITEMS	QUANTITY	COST (KSH)/UNIT	AMOUNT
Laboratory detection of brucella and leptospira pathogens		1851/sample	275,000
Cost of hiring biomedical freezer and refrigerator for storage of blood samples and processed DNA materials.	2 for 90 days	1000/day	90,000
Consumables for packing and storing samples (cool boxes, vacutainer tubes and needles for blood collection, racks)			100,000
Cost of hiring translators in the field	1 translator for 30 days	2000/day	20,000
Cost of travelling to the field for data collection	1individual for 30days	1000/day	90,000
Cost of accomodation for data collection	1individual for 30days	2000/day	100,000
Cost of hiring laboratory technicians	1technician for 20 days	2000/day	40,000
Contigency allowance			20,000
TOTAL			625,000

UNIVERSITY OF NAIROBI

Declaration of Originality Form

Name of the student:	Amos Sibuti Mwasi
Registration Number:	J56/14130/2018
College:	College of Agriculture and Veterinary Sciences
Faculty/School/Institute:	Veterinary Medicine
Department:	Public Health, Pharmacology and Toxicology
Course Name:	Veterinary Epidemiology and Economics
Title of the work:	Serological and molecular detection of Brucellosis and its risk factors for sheep and goat flocks in Kajiado County, Kenya.

DECLARATION

I understand what Plagiarism is and I am aware of the University's policy in this regard.

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of degree or publication. Where other people's work or my own work has been used, this has been properly acknowledged and referenced in accordance with University of Nairobi's requirements.

I have not sought or used the services of any professional agencies to produce this work.

I have not allowed and shall not allow anyone to copy my work with the intention of passing it off as his or her own work.

I understand that any false claim in respect of this work shall result in disciplinary action in accordance with University Plagiarism Policy.

Signature………⟨signature⟩…………**Date**........3rd November, 2021…….

Student ID

University of Nairobi
A world-class university committed to scholarly excellence

| Portal Home | Student Fees | Timetables | Course Registration | Results | Enquiries | Book Room | Logout |

Change Password · My profile · Year 1 Registration · Student ID · Inter Faculty · Clearance Status · Caution Refund
Academic Tracking

J56/14130/2018 AMOS SIBUTI MWASI (Regular/Integrated)

| ID/PP No. | | Type : New (First Time) ∨ | Make Request |

Previous Requests

	Request Date	Status	Receipt No.	Validity	Remarks	
1.	09-NOV-2018	PRINTED 29-NOV-2018 04:08		29-NOV-2018 - 29-NOV-2019	ID Already Printed	1.
2.	27-NOV-2019	PRINTED 11-DEC-2019 11:19		11-DEC-2019 - 10-DEC-2020	ID Already Printed	2.
3.	16-JUN-2021	PENDING			ID Card Available for Printing.	3.

Procedure for getting the new generation Student ID Card

1. Ensure that your fees (including that of Student ID) is paid and receipted before making the ID card request.

2. Fees for Re-Issue of lost ID card must be paid and receipted separately.

3. Place your request for the Student ID through the Student Portal.

4. Request for renewal of expired ID card should be made **NOT MORE THAN ONE MONTH BEFORE EXPIRY OF THE CURRENT ONE** .

5. Ensure that your photo has been taken and uploaded into the System.The University Photographer is located at JKML Library.

6. Allow at least two working days for the processing of your ID card.

7. Keep checking the status of your ID request through the Student Portal.

8. Collect your printed Student ID from your Faculty / School / Institute Office once the STATUS of your request is reflected as PRINTED.

Note:

1. Validity for Re-Issued ID Card will be the same as that of previously Issued (Lost) ID.

2. Validity for Replacement / ID Re-New will start after expiry of current Issued ID.

About Us ·

Plagiarism report

SEROLOGICAL AND MOLECULAR DETECTION OF BRUCELLOSIS
AND ITS RISK FACTORS FOR SHEEP AND GOAT FLOCKS IN
KAJIADO COUNTY, KENYA

ORIGINALITY REPORT

11%	8%	8%	4%	3/11/2021
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS	

PRIMARY SOURCES

1	Submitted to Mansoura University Student Paper	2%
2	zelsbrucellosis.files.wordpress.com Internet Source	1%
3	Cristina Ballesteros, Amos Mwasi, Eunice Mungai, Awo Ibarahim et al. "Developing and validating a rapid assessment tool for small ruminant reproduction and production in pastoralist flocks in Kajiado, Kenya", Veterinary and Animal Science, 2021 Publication	1%
4	tjpr.org Internet Source	1%
5	www.ncbi.nlm.nih.gov Internet Source	1%
6	Erepository.uonbi.ac.ke Internet Source	1%
	worldwidescience.org	

PROF J.D.MANDE

DEAN, FVM 12/11/2021

Dean
Faculty of Veterinary Medicine
University of Nairobi
29053-00625, NAIROBI

65

Fee statement

University of Nairobi
A world-class university committed to scholarly excellence

Portal Home   Student Fees   Timetables   Course Registration   Results   Enquiries   Book Room   Logout

Fee Statement   Detailed Fee   Pay Fees Online
Statement

J56/14130/2018 AMOS SIBUTI MWASI   (Regular/Integrated)

Fees Statement

Academic Year : 2018/2019   Billing Currency : KES

Transaction/ Receipt Number	Date	Description	Debits DR	Credits CR	Balance	Cur.Rate
2180324386	2018-11-06	FEES PAYMENTS	0.00	22,500.00	-22,500.00	KES=1
J56/14130/2018-2018/2019-239/TUIT	2018-11-05	TUIT WAIVER	0.00	185,000.00	-207,500.00	KES=1
J56/14130/2018-2018/2019-SEM1	2018-11-16	FEES PAYABLE FOR SEM1	121,500.00	0.00	-86,000.00	KES=1
J56/14130/2018-2018/2019-SEM2	2019-01-22	FEES PAYABLE FOR SEM2	100,000.00	0.00	14,000.00	KES=1
		Academic Year Totals :	221,500.00	207,500.00	14,000.00	

Closing Balance : 14,000.00

Academic Year : 2019/2020

Transaction/ Receipt Number	Date	Description	Debits DR	Credits CR	Balance	Cur.Rate
	Opening Balance		14,000.00	0.00	14,000.00	
2180441223	2019-10-29	FEES PAYMENTS	0.00	16,500.00	-2,500.00	KES=1
J56/14130/2018-2019/2020-239/TUIT	2019-11-05	TUIT WAIVER	0.00	115,000.00	-117,500.00	KES=1
J56/14130-2019/2020-SEM1	2021-08-12	FEES PAYABLE FOR SEM3	117,000.00	0.00	-500.00	KES=1
J56/14130/2018-PV 62824/1 BOOK ALLOWANCE	2019-11-26	REFUNDS	6,000.00	0.00	5,500.00	KES=1
J56/14130/2018-PV62993/7 RESEARCH ALLOW	2019-12-10	REFUNDS	18,000.00	0.00	23,500.00	KES=1
		Academic Year Totals :	155,000.00	131,500.00	23,500.00	

Closing Balance : 23,500.00

Academic Year : 2021/2022

Transaction/ Receipt Number	Date	Description	Debits DR	Credits CR	Balance	Cur.Rate
	Opening Balance		23,500.00	0.00	23,500.00	
J56/14130/2018-FV 62824/1 BOOK ALLOW ACC	2021-08-09	PV 62824/1 BOOK ALLOW ACC	0.00	6,000.00	17,500.00	KES=1
J56/14130/2018-PV62993/7 RESEARCH ALLOW ACC	2021-08-09	PV62993/7 RESEARCH ALLOW ACC	0.00	18,000.00	-500.00	KES=1
		Academic Year Totals :	23,500.00	24,000.00	-500.00	

Closing Balance : -500.00

About Us

© 2013 University of Nairobi . Design: by ICT Centre

GOVERNMENT-SPONSORED (MODULE I) STUDENTS PAYMENT INSTRUCTIONS

1. Bank Account
=> Cash Deposits, EFT or RTGS transfer to UON MODULE I Collection Account No. 2032770838 at ABSA Bank, Plaza Branch
2. M-Pesa Pay Bill
=> The Business Number is 503003
=> The Account Number is your "Student Registration Number"

SELF-SPONSORED PROGRAMMES (MODULE II) PAYMENT INSTRUCTIONS / OPTIONS

1. Bank Account
=> Cash Deposits, EFT or RTGS transfer to UON MODULE II Collection Account No. 2032771362 at ABSA Bank, Plaza Branch
=> Cash Deposits, EFT or RTGS transfer to UON US$ Dollar Account No. 2032770625 at ABSA Bank, Plaza Branch
2. M-Pesa Pay Bill
=> The Business Number is 300059
=> The Account Number is your "Student Registration Number" (or "Admission Ref Number" for new student)
NOTE: CASH, AGENCY BANKING AND ATM DEPOSITS ARE NOT ALLOWED

https://smis.uonbi.ac.ke/statement_summary.php

1/1

UNIVERSITY OF NAIROBI DIGITAL REPOSITORY

DEPOSIT AGREEMENT

To efficiently administer the University of Nairobi Digital Repository and preserve its contents for long-term use, the University requires certain permissions and warrants from a depositor or copyright owner. By accepting this agreement, a copyright owner still retains copyright to their work and does not give up the right to submit the work to publishers or other repositories. If one is not a copyright owner, they represent that the copyright owner has given them permission to deposit the work.

By accepting this agreement, a depositor/copyright owner grants to the University the non-exclusive right to reproduce, translate and distribute the submission, including the descriptive information (metadata) and abstract , in any format or medium worldwide and royalty free, including, but not limited to, publication over the internet except as provided for by an addendum to this agreement.

By depositing my/our work in the University of Nairobi Digital Repository, I agree to the following:

(i)   This submission does not, to the best of my/our knowledge, infringe on anyone's copyright or other intellectual property rights.

(ii)   If the submission contains material for which I/we do not hold copyright and that exceeds fair use, I/we have obtained the unrestricted permission of the copyright towner to grant the University the rights required by this agreement and that such third-party owned material is clearly identified and acknowledged within the text or content of the submission

(iii)   The submitted material does not contain any confidential information, proprietary information of others or export controlled information

(iv)   There are no restrictions or required publication delays on the distribution of the submitted material by the University

(v)   Once the submission is deposited in the repository, it remains there in perpetuity

(vi)   The information I/we provide about the submitted material is accurate

(vii)   That if copyright terms for, or ownership of, the submitted material changes, it ismy/our responsibility to notify the University of these changes

I/we understand that the University of Nairobi Digital Repository:

(i)   May make copies of the submitted work available world-wide, in electronic form at/via any medium for the lifetime of the repository, or as negotiated with the repository administrator, for the purpose of open access

(ii)   May electronically store, translate, copy or re-arrange the submitted works to ensure its future preservation and accessibility within the lifetime of the repository unless notified by the depositor that specific restrictions apply

(iii)   May incorporate metadata or documentation into public access catalogues for the submitted works. A citation/s to the work will always remain visible in the repository during its lifetime

(iv)   Shall not be under any obligation to take legal action on behalf of the depositor or other rights holders in the event of breach of intellectual property rights or any other right in the material deposited

(v)   Shall not be under any obligation to reproduce, transmit, broadcast, or display the submitted works in the same format or software as that in which it was originally created

(vi)   May share usage statistics giving details of numbers of downloads and other statistics with University of Nairobi staff

While every care will be taken to preserve the submitted work, the University of Nairobi is not liable for loss or damage to the work(s) or associated data while it is stored within the digital repository.

Work(s) to be deposited:

Title: Serological and molecular detection of Brucellosis and it's risk factors in sheep and goat flocks in Kajiado county, Kenya.

Author: Amos Sibuti Mwasi

Depositor's Declaration

I Amos Sibuti Mwasi

hereby grant to the University of Nairobi Digital Repository, a non-exclusive license on the terms outlined above.

Name: Amos Sibuti Mwasi

College: College of Agriculture and Veterinary Services

Sign:

Date: 10th October, 2021