INVESTIGATION OF *TOXOPLASMA GONDII* AND ASSOCIATED RISK FACTORS FOR INFECTION OF SHEEP AND GOATS IN KAJIADO COUNTY

BY

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DECLARATION

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

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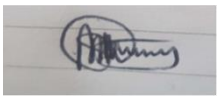
Signature:  Date: ……03/11/2021……..

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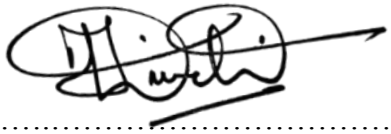
Signature:  Date: 10th November 2021

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DEDICATION

I dedicate this thesis to the glory of the almighty God

To Sammy, Claire, Cynthia and Alex

and

In memory of my late parents

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LIST OF ABBREVIATIONS AND ACRONYMS

A	Adenine
Bp	Base pair
C	Cytosine
CNS	Central nervous system
DAT	Direct agglutination test
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
ELISA	Enzyme linked immunosorbent assay
EDTA	Ethylenediaminetetraacetic acid
G	Guanine
GoK	Government of Kenya
LAT	Latex agglutination test
MAT	Modified agglutination test
mg	Milligram
mM	Millimolar
Min.	Minute
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
T	Thymine
µl	Microlitre

ABSTRACT

Toxoplasma gondii has significant veterinary and public health importance globally. Infection in sheep and goats reduces their reproductive performance and may serve as a source of infection to humans leading to a life-threatening infection especially among the immunosuppressed individuals. In spite of this, *Toxoplasma gondii* in sheep and goats has not been expansively studied in Kenya. A cross- sectional study was conducted between the months of June and November 2019 in five selected wards in Kajiado County, Kenya. These included Kenyawa Poka, Ildamat, Iloodokilani, Matapato South and Kaputei North wards. The objective was to determine prevalence of *Toxoplasma gondii* infection in sheep and goats using serological and molecular methods and to analyze risk factors for its occurrence and the potential exposure factors for human infection.

A structured questionnaire was administered to 130 randomly selected pastoralists' households whose sheep and goats were recruited in the study in order to collect data on variables such as flock sizes, source of water, presence of cats, reproductive history, neonatal mortality, type of production system, consumption of raw meat, milk and blood. A total of 1,464 serum samples from female animals; sheep (n=842) and goats (n=622) in 122 flocks, were tested for anti-*Toxoplasma gondii* antibodies using Indirect Enzyme-Linked Immunosorbent Assay (ELISA). Furthermore, sero-positive samples were tested for the presence of *Toxoplasma gondii* DNA material in blood using conventional Polymerase Chain Reaction (PCR).

The data were entered in Microsoft excel® spreadsheet and analyzed using SPSS® statistical package version 25. The prevalence of *Toxoplasma gondii* was 9.0% (76/842; 95% CI: 7.3-11.2) in sheep and 12.5% (78/622; 95% CI: 10.2-15.4) in goats. The prevalence was statistically different between the sheep and goats (95% CI: 0.003-0.068; $P = 0.0337$). *Toxoplasma gondii* DNA was detected in 88.96% (137/154; 95% CI: 83.0-93.0) of the seropositive sheep and goats.

There were no statistically significant flock level risk factors to *Toxoplasma* positivity in sheep and goats identified in this study. However, the potential exposure factors to human infection with *Toxoplasma gondii* in Kajiado County included failure to use gloves while handling aborted materials from livestock, consumption of raw blood, raw meat, and unpasteurized milk, and pastoralists' lack of knowledge on zoonotic diseases.

This study concluded that *Toxoplasma gondii* is prevalent in sheep and goats in the study area with a prevalence of 9.0% in sheep and 12.5% in goats. This finding implied that toxoplasmosis is likely to be one of the contributing factors to reproductive problems in sheep and goats in the study area. The presence of *Toxoplasma gondii* in livestock presents a potential threat to health of pastoralist community in Kajiado County.

This study recommends the need for public health education to raise awareness on toxoplasmosis in order to reduce its transmission between man and livestock. Subsequently, it is recommended that further research need to be focused towards determination of prevalence of this parasite in other livestock, the extent of environmental contamination and assessment of the impact of this parasite on human and animal health. This study also recommends more research on neglected zoonotic diseases in the country as part of One Health approach.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Study background

This study focused on *Toxoplasma gondii*, an obligatory intracellular protozoa which belongs to the subclass Coccidia, order Eucoccidiorida and family Sarcocystidae (Dhaliwal and Juyal, 2013). Its definitive hosts are members of the feline family while mammals, birds and reptiles are intermediate hosts (Dubey, 2009). It causes toxoplasmosis, a prevalent protozoan zoonotic disease with great veterinary significance (Tonouhewa *et al.,* 2017). Toxoplasmosis has the highest prevalence among parasitic diseases affecting man (Mose *et al.,* 2016). It is the most important protozoal zoonotic disease known to cause abortion in animals and humans worldwide (Shaapan, 2015). In sheep and goats, the disease causes reproductive failure through early embryonic death and resorption, fetal death and mummification, abortions, stillbirths as well as neonatal mortality that results into economic losses to the farmers (Tilahun *et al.,* 2018). Other zoonotic protozoal diseases associated with abortion include sarcosporidiosis, neosporosis and trypanosomiasis (Shaapan, 2015). Oocysts in the environment are the main sources of infection for sheep and goats whereas in man, the main source of infection is hypothesized to be due to interaction between humans, animals and animal products (Glor *et al.,* 2013).

Sheep, goats and pigs are reported to have the highest incidence of *Toxoplasma gondii* cysts in meat and play an important role in human infection (Glor *et al.,* 2013). In humans, the disease causes abortion, neonatal death or severe birth defects such as retinochoroiditis and blindness or life threatening complications such as meningoencephalitis especially among the immune-compromised individuals (Tonouhewa *et al.,* 2017). Therefore, toxoplasmosis has important public health implications in society.

1.2 Statement of the Research problem

Toxoplasmosis reduces productivity in sheep, goats and pigs. It is a public health concern due to its zoonotic nature. In Kenya, scanty published data exists on *Toxoplasma gondii* in sheep and goats and the associated risk factors for infection. Abwajo, (1984) is the only publication on seroprevalence in sheep and goats in the country. Several studies in Africa, for instance, in Ethiopia (Tilahun *et al.,* 2018), South Africa (Samra *et al.,* 2007), Tunisia (Lahmar *et al.,* 2015) and Sudan (Atail *et al.,* 2017) have reported a varied prevalence of the disease in sheep and goats based on serological analysis. However, scanty information exists on detection of *Toxoplasma gondii* using molecular methods. Furthermore, only a few studies have investigated the infection in humans. This has made toxoplasmosis to be one of the neglected zoonotic diseases in the country (Mbabu *et al.,* 2014 and Munyua *et al.,* 2016). These represented significant research gaps. In Kenya, detection of *Toxoplasma gondii* in these animals expounded a risk for human infection within the study area where consumption of poorly cooked or raw meat and milk are prevalent, livestock products and wastes are handled without precautionary measures and there is interaction between livestock and the domestic and wild felids.

1.3 Justification of the study

It was essential to clarify the existence of *Toxoplasma gondii* and identify the associated risk factors for infection of sheep and goats in the study area in order to create awareness and support the development of an effective control strategy in animals. Furthermore, the findings would also be useful for further research in humans. This study also contributes immensely to the understanding of toxoplasmosis. The findings are valuable to veterinarians, physicians, public health implementers, policy makers, researchers and other beneficiaries in prioritizing the surveillance, reporting, planning and implementation of preventive and control strategies of the disease in Kenya.

1.4 Study hypothesis

Toxoplasma gondii is a common parasite affecting sheep and goats in Kajiado County and there exists some potential risk factors contributing to occurrence of this parasite in these animals and humans.

1.5 General objective

To investigate the presence of *Toxoplasma gondii* and its associated risk factors for infection of sheep and goats in Kajiado County

1.5.1 Specific objectives

1. To determine the prevalence of *Toxoplasma gondii* in sheep and goats using serological and molecular methods in Kajiado County
2. To analyze the risk factors associated with *Toxoplasma gondii* infection in sheep and goats and potential exposure factors to humans in Kajiado County

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Toxoplasma gondii*

Toxoplasma gondii is a protozoan organism first discovered by two scientists; Charles Nicolle and Louis Manceaux in 1908 from tissues of a rodent in Tunis (Dubey, 2009). It is classified taxonomically under the phylum Apicomplexa, class Conoidasida, order Eucoccidiorida, family Sarcocystidae and genus *Toxoplasma* (Shaapan, 2015).

Toxoplasma gondii is an obligate intracellular cyst-forming protozoan organism affecting all mammals and birds as intermediate hosts but cats and other felines act as the definitive hosts (Glor *et al.,* 2013). The parasite has the broadest range of hosts and widest geographic distribution among all animal and human parasites (Ahmad, 2014). It is known to be a very efficacious parasite globally (Djurković-Djaković, *et al.,* 2019). It causes toxoplasmosis, an asymptomatic disease in many animals but life threatening in a few that acquire acute infection characterized by hepatomegaly, lymphadenopathy, nervous signs and interstitial pneumonia; stillbirths, abortion, weak offsprings, or mummification of foetus in pregnant animals (OIE, 2012b).

Toxoplasma gondii exists in three forms; sporozoites found in mature oocysts, tachyzoites and bradyzoites that exists as tissue cysts (Robert-Gangneux and Dardé, 2012). Sporulated oocysts have two sporocysts, each containing four sporozoites (OIE, 2012b). *Toxoplasma gondii* is classified into 15 haplogroups congregated into six clades and in which are three clonal lineages (type I, II and III) and 189 other genotypes such as Africa 1 in Africa, Chinese 1 in Asia; BrI, BrII, BrIII, BrIV in Central and South America and Type 12 in North America (Innes *et al.,* 2019). Among the clonal lineages, type I is known to be the most virulent and multiplies faster than type II and III (Gebremedhin, 2014 and Hanafiah *et al.,* 2018) whereas type II is

predominantly known to cause disease in humans while type I and III infect animals (OIE, 2012b).

2.2 *Toxoplasma gondii* life cycle and transmission

The life cycle of *Toxoplasma gondii* consists of an intraintestinal sexual reproduction stage occurring only within the small intestines of the felidae family resulting into development of its characteristic oocysts (Gebremedhin *et al.,* 2015). The oocysts sporulate in the environment over a period of 1–5 days under adequate aeration, humidity and temperature to become infective and may remain infective for a year or more since they are very resistant to environmental conditions (OIE, 2012b). Upon ingestion by a susceptible animal, the sporulated oocyst releases the sporozoites that penetrate the intestinal lining, become tachyzoites and establish an infection (OIE, 2012b). These tachyzoites transform into bradyzoites which are more prevalent in neuronal and muscle tissues especially the eyes, brain, heart and skeletal muscles (Dubey *et al.,* 1998). Other organs such as the lungs, liver and kidneys are also affected and this constitutes the extraintestinal asexual reproduction stage of the parasite in cats and other intermediate hosts (Gebremedhin, 2014). In domestic animals, these tissue cysts are normally found in many tissues of parasitized sheep, goats and pigs; less often in rabbits and poultry, and rarely in cattle (Tenter, 2009). Tissue cysts can persist for years without eliciting an inflammatory reaction (Dubey *et al.,* 1998).

Dubey, (2004) and Daka, (2014) reported that *Toxoplasma gondii* is transmitted horizontally via ingestion of pasture, water, fruits and vegetables contaminated with oocysts or meat with tissue cysts as well as vertically via transplacental transfer of tachyzoites in both animals and man. Rouatbi *et al.,* (2019) reported that transmission of *Toxoplasma gondii* in man is majorly through ingestion of oocysts shed by felines or tissue bradyzoites in raw or under-cooked meat, and

vertical transmission that occurs when tachyzoites cross the placenta to the growing fetus in pregnant women as shown in figure 1 (Deng *et al.,* 2021). It can also be transmitted through blood transfusion and organ transplantation (Shapaan, 2015) and occupational accidental inoculation of tachyzoites (Mose *et al.*, 2020).

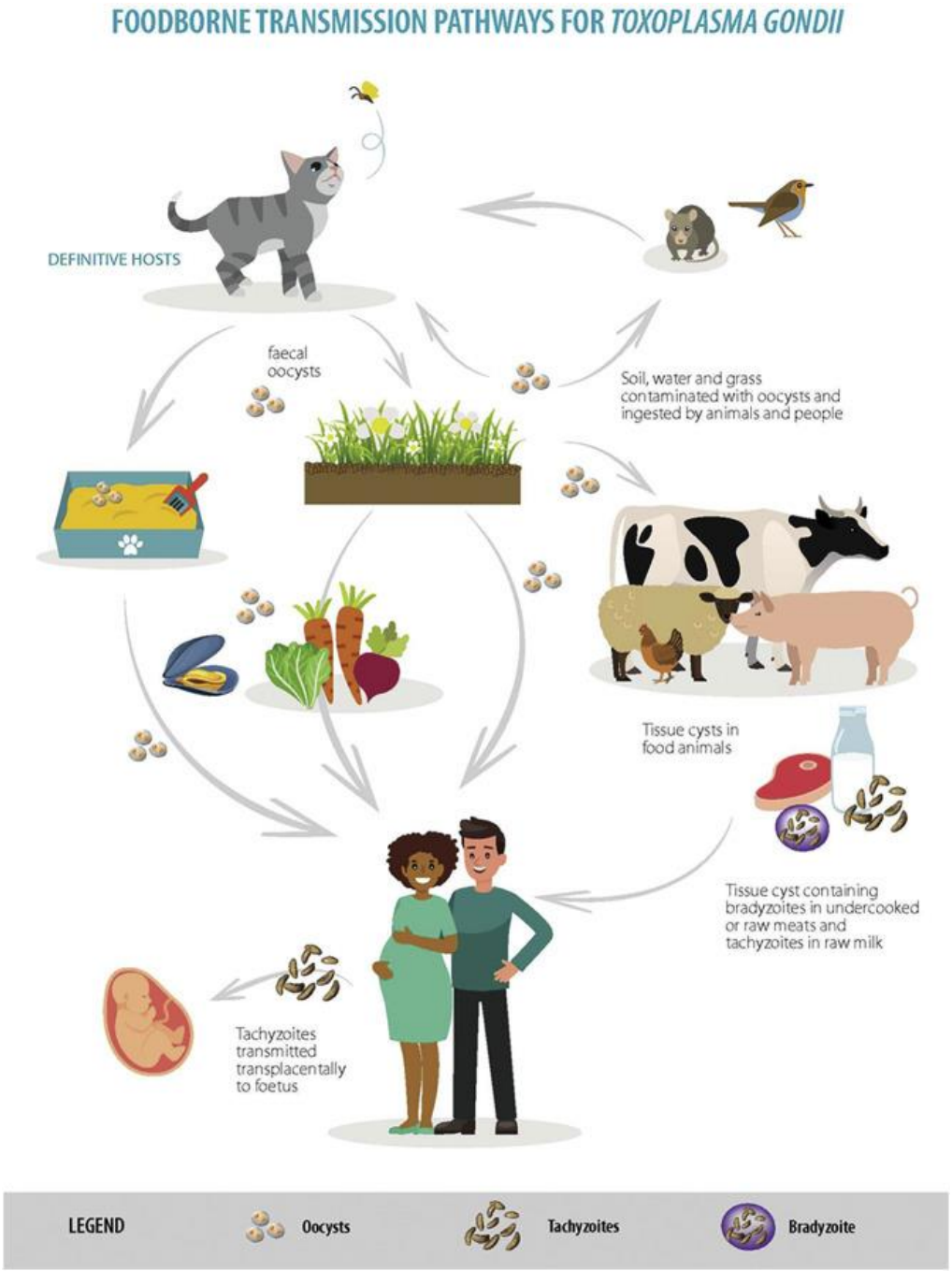

Figure 1: Life cycle of *Toxoplasma gondii.* Source: Adopted from Deng *et al.*, (2021).

Mechanical vectors such as cockroaches, earthworms and flies have been reported to disseminate oocysts while dogs have been reported to shed oocysts in faeces that can contaminate food and water of intermediate hosts (Shapiro *et al.,* 2019).

2.3 Economic importance of *Toxoplasma gondii*

This parasite causes toxoplasmosis, a sporadic disease which is among the leading causes of reproductive failure in animals through embryonic or fetal death, abortions, stillbirths and neonatal mortality (Ahmad, 2014 and Atail *et al.,* 2017). This culminates into increased cost of production, reduced milk production, reduced number of replacement stock and stagnation of genetic advancement (Gebremedhin *et al.,* 2014). These were estimated to lead to loss of £11 million in the United Kingdom and $1.4 to 4.7 million in Uruguay annually in the sheep industry (Tilahun *et al.,* 2018). In man, toxoplasmosis causes severe birth defects and has been linked to epilepsy, schizophrenia, road accidents and death due to toxoplasmic encephalitis especially among the immune-compromised people (Tonouhewa *et al.,* 2017). Investigation of the economic impact of toxoplasmosis in Kenya is a potential area for further studies.

2.4 Prevalence of *Toxoplasma gondii*

Toxoplasmosis is neglected and under-reported in many parts of the world but is known to be of low prevalence in hot and arid climates (Gebremedhin *et al.,* 2014). In domestic ruminants, a global seroprevalence of 30% in sheep, 15% in goats and 9% in cattle has been estimated by Samra *et al.,* (2007). According to a review and meta-analysis of serological studies done in Africa, the seroprevalence of *Toxoplasma gondii* was reported to be 26.1% in sheep, 22.9% in goat, 12% in cattle, 36% in camel, 26.0% pig and 37.4% in chicken (Tonouhewa *et al.,* 2017).

In Kenya, Abwajo**,** (1984) reported a seroprevalence of 56% in sheep and 21% in goats. Molecular methods such as nested Polymerase Chain Reaction (nPCR) have been used to

determine 7.8% prevalence in cats by Njuguna *et al.,* (2017) while Mose *et al.* (2016) detected 79% prevalence in free-range chickens using the same method. The molecular prevalence of *Toxoplasma gondii* and the risk factors for infection in sheep and goats as well as seroprevalence in other livestock have not been established in Kenya and these represented major knowledge gaps that needed to be investigated.

Other studies have reported that a third of the world human population is seropositive for *Toxoplasma gondii* (Tonouhewa *et al.,* 2017). In Kenya, the prevalence of toxoplasmosis in humans has been estimated to range from 23-60% (Mose *et al.,* 2016). In addition, 39% of slaughterhouse workers (Thiongo *et al.,* 2016) and 54% of blood donors (Ogendi *et al.,* 2013) have been reported to be infected with *Toxoplasma gondii*.

2.5 Clinical manifestation of *Toxoplasma gondii* infection in sheep and goats

Toxoplasmosis is known to cause embryonic death and resorption, fetal death and mummification, abortion, stillbirths, encephalitis, pneumonia, weak births and neonatal mortality in sheep and goats (Ahmad, 2014 and Atail *et al.,* 2017). In Europe, 11–24% of aborted ovine fetuses have been found hosting *Toxoplasma gondii* DNA (Tzanidakis *et al.,* 2012). The characteristic feature in abortion caused by *Toxoplasma gondii* are focal whitish spots of necrosis in placental cotyledons (Shapaan, 2015). Acute toxoplasmosis is fatal in young animals and the infection is known to cause encephalitis, enteritis, nephritis, and hepatitis in adult goats (Ahmad, 2014).

2.6 Immunity

Toxoplasma gondii stimulates production of antibodies by B-cells where IgM is the first to appear which peaks after two weeks followed by IgA and IgE while IgG peaks after four months (Daka, 2014). Detection of IgM is a pointer towards a recent or current infection (Paul *et al.,*

2018). IgG persists at low levels for a lifetime in immunocompetent individuals (Verma and Khanna, 2013) . However, protective immunity in animals involves a cellular response of T-cells (CD4+ and CD8+) and interferon gamma (IFN-γ) since *Toxoplasma gondii* is obligate intracellular parasite within a parasitophorus vacuole (Dubey, 2009).

2.7 Risk factors for *Toxoplasma gondii* infection in sheep and goats

The prevalence of toxoplasmosis varies depending on several factors such as age and sex of the animal, animal species, presence of cats, flock size among other factors (Ahmad *et al.*, 2015).

2.7.1 Age

Tonouhewa *et al.,* (2017 observed that most serological studies involving sheep and goats in Africa reported a high seropositivity in aged animals. Dubey (2009) reported that the likelihood of being infected with *Toxoplasma gondii* is absolutely associated with the age of sheep. Tegegne *et al.,* (2016) reported a higher prevalence in animals more than one year old whereas Lahmar *et al.,* (2015) reported a higher prevalence in animals more than three years old. Ahmad *et al.,* (2015) found that animals older than 36 months had a higher prevalence and attributed this to the fact that older animals are exposed to other risk factors for infection for longer periods and from different sources compared to the young animals. However, Hanafiah *et al.,* (2018) found that young cats have a higher prevalence of oocyst compared to older ones, therefore, age is an imperative risk factor.

2.7.2 Sex

Female sheep and goats were found to have a higher prevalence than males suggesting that females are more susceptible to toxoplasmosis than males (Tegegne *et al.,* 2016). This is probably because the immunity is lowered by poor nutrition, pregnancy, lactation, age,

environmental factors among other factors in females (Ahmad *et al.,* 2015 and Mose *et al.,* 2016).

2.7.3 Species

Sheep have been found to have a higher prevalence of toxoplasmosis compared to goats (Ahmad, 2014). For instance, Gebremedhin *et al.,* (2014) reported a 20% and 15% prevalence in sheep and goats respectively in Ethiopia using DAT. They attributed the higher prevalence in sheep to feeding habits whereby sheep are grazers and more likely to be infected from oocysts contaminated pastures than goats who are browsers. However, Ahmed *et al.,* (2016) and Gharekhani *et al.,* (2018) have reported a higher prevalence in goats than sheep using ELISA in Pakistan and Iran respectively. Ahmed *et al.,* (2016) attributed this to the higher activity and movement in goats than sheep that increases their exposure to *Toxoplasma gondii* in the environment. Contaminated outdoor water sources have been recognized to be a high risk for toxoplasmosis infection in goats (Ahmad *et al.,* 2015). Tonouhewa *et al.,* (2017) attributed the difference between infection in various species to disparity in number of domestic and wild cats around farms, climatic factors, management practices as well as the cut-off titer, the sample size, sensitivity difference in the serological tests used and the duration of various studies carried out in Africa. Dubey, (2009) recommended further studies to clarify on this difference.

2.7.4 Breed

There are breed differences in infection with toxoplasmosis but the reasons have not been fully understood (Dubey, 2009). For instance, a study done in Tunisia suggested that Barbarine breed of sheep were more susceptible to toxoplasmosis than other breeds of sheep in that country (Lahmar *et al.,* 2015). Similarly in Pakistan, salt range breed of sheep was found to be more

susceptible than other breeds and this was attributed to overstocked extensive management (Ahmad *et al.,* 2015).

2.7.5 Presence of cats

Cats are the definitive hosts of *Toxoplasma gondii* and their presence in the same environment with sheep and goats has been recognized as a risk for infection since they shed oocysts in their faeces thus take part in transmission to other animals including man through contamination of pastures and water (Ahmad *et al.,* 2015). Globally, a seropositivity of 60% has been estimated in cats (Muhie and Keskes, 2014). Njuguna *et al.,* (2017) reported 7.8% prevalence of *Toxoplasma gondii* in domestic cats in Thika region of Kenya. It has been documented that few cats are adequate to contaminate an extensive area as one infected cat is capable of shedding millions of oocysts (Tilahun *et al.,* 2018).

2.7.6 Flock size

Flock sizes larger than 50 individuals in sheep and 30 in goats were found to have a higher seroprevalence in Pakistan and this made larger flocks to be reared under semi-intensive or extensive system rather than intensive system hence had a higher chance of getting infected due to increased exposure to contaminated environment with cat faeces (Ahmad *et al.,* 2015). Contrary to this report, small flock size was found to be a risk factor for infection in sheep in Italy (Cenci-Goga *et al.,* 2013).

2.7.7 Production system

Higher seroprevalence of toxoplasmosis has been recorded more in extensively managed sheep systems in Ghana, Pakistan, Brazil and China since the probability of ingesting oocysts contaminated food and water is higher than under semi-intensive and intensive systems (Tonouhewa *et al.,* 2017). The inverse was true in a study done in South Africa in sheep but this

phenomenon was attributed to dry climatic conditions hence extensive system was observed to have a lower prevalence (Samra *et al.,* 2007). Seroprevalence in intensively managed sheep has been reported to be lower than in sheep under semi-intensive management (Dubey, 2009).

2.7.8 Source of drinking water and water quality

Oocysts need a humid and warm environment to survive and can remain viable for an indefinite period in water and therefore, drinking water contaminated with cat feces predisposes susceptible animal hosts to *Toxoplasma gondii* (Tilahun *et al.,* 2018). Contaminated outdoor water sources have been recognized to be a high risk for toxoplasmosis infection in goats (Ahmad *et al.,* 2015). Stray cats accessing the animals' water was found to be a risk factor for infection in sheep (Cenci-Goga *et al.,* 2013).

2.7.9 Environmental contamination

Soil is contaminated with *Toxoplasma gondii* oocysts after infected felids shed them in their feces and once sporulated, can resist temperature up to 35ºC thus exposure to such soil is one of the risk factors for human and animal infection (Shapiro *et al.,* 2019). Seroprevalence of toxoplasmosis has been found to be higher in animals kept under poor hygienic conditions that favor food and water contamination with cat faeces (Ahmad *et al.,* 2015).

2.8 Risk factors for *Toxoplasma gondii* infection in humans

Consumption of undercooked meat has been reported as one of the principal risk factor for *Toxoplasma* infection in humans by Muhie and Keskes (2014). Glor *et al.,* (2013) reported that animal infection is the main source of human infection and that sheep and goats serve as the key source of infection in man. The transmission of toxoplasmosis in man mainly depends on an environment where culture and other beliefs influence the eating habits (Tenter, 2009 and Gebremedhin *et al.,* 2015). Intake of raw or undercooked meat such as lamb and mutton or meat

products containing *Toxoplasma gondii* tissue cysts has been reported in numerous studies as the main source of human infection (Tonouhewa *et al.,* 2017). In an earlier study in Kenya, it was expounded that consumption of raw meat, raw blood and unpasteurized milk as well as handling infected aborted matter without protective gear are probable sources of exposure to zoonoses (Onono *et al.,* 2019). Chege *et al.,* (2015) highlighted that among the Maasai community, fermenting and consumption of raw milk mixed with blood is inherent and that animal parts commonly eaten raw include kidneys, the liver and fatty tails of sheep. Tilahun *et al.,* (2018) reported that the risk factors for human toxoplasmosis include possession of cats, cleaning cats' litter boxes and making contact with soil contaminated with cat faeces without gloves and consumption of unwashed vegetables and fruits leading to ingestion of sporulated oocysts. Ahmad, (2014) reported that risk factors in humans that were considerably linked with seroprevalence were source of livelihood, low level of education, eating of mutton and preference for raw or undercooked meat, consumption of unpasteurized milk from infected animals, out-of-doors water sources, contact with soil and presence of cats in environment. Consumption of unpasteurized goat milk has also been incriminated (Gebremedhin, 2014).

2.9 Diagnosis

It is difficult to diagnose toxoplasmosis by use of clinical signs due to their non-specific nature in both animals and man (Ahmad, 2014). The conclusive diagnosis of toxoplasmosis is principally by identification of the presence of the *Toxoplasma gondii* or its antibodies by molecular, parasitological and serological tests where each technique has its own sensitivity and specificity, depending on tissue or sample to be analyzed (Mose *et al.,* 2016). Serological methods are most widely used for diagnosis of *Toxoplasma gondii* (Thiongo *et al.,* 2016).

2.9.1 Indirect or serological methods

Serological methods depend on detection of antibodies against the parasite and the methods are fast, highly sensitive and globally useful for diagnosis (Glor *et al.,* 2013). They include Enzyme-linked Immunosorbent Assay (ELISA), the Sabin–Feldman Dye Test (DT), Modified Agglutination Test (MAT), Indirect Fluorescent Antibody Test (IFAT), Western Blot (WB), Latex Agglutination Test (LAT) and Indirect Hemaglutination Test (IHA) (Glor *et al.,* 2013). The most commonly used serological tests are MAT and ELISA (Glor *et al.,* 2013). The ELISA test is simple, inexpensive and suitably useful in field conditions and can be modified to detect *Toxoplasma gondii* specific IgM, IgG and IgA antibodies as well as antigens in the body.

It is reported that ELISA for detecting *Toxoplasma gondii* IgG and IgM antibodies in serum in man and animals has a high sensitivity and specificity (Ahmad, 2014). Muhie and Keskes (2014) reported that detection of IgM antibodies is associated with clinical toxoplasmosis but failure to detect them does not eliminate it. IgG antibodies persist for years post-infection but a fourfold rise in titers indicates recent infection (Muhie and Keskes, 2014).

The indirect ELISA is carried out as described by Ahmad, (2014): Serum is diluted up to 400 times and placed into the antigen coated plates where antibodies, if present in serum, bind to the antigen coated plate during incubation. Washing of the plate is done to eliminate unbound material and then a second enzyme-linked antibody is applied which bind to the preceding antibody. A substrate is then added after washing the plate. A colour is produced whose optical density is measured by spectrophotometer if antigen-antibody reaction occurs. Drawbacks to serological methods in diagnosing toxoplasmosis include low antibody levels especially during early infection leading to low sensitivity and lengthy and laborious test procedures (Lau *et al.,* 2010 and Gebremedhin, 2014).

2.9.2 Molecular methods

Molecular methods such as Polymerase Chain Reaction (PCR) have developed into indispensable and reliable tools since they are extremely sensitive, highly specific and fast compared to serological tests since they do not depend on the immune status of the animals (Liu *et al.*, 2015 and Mose *et al.*,2016). Samples include blood, amniotic fluid, cerebrospinal fluid, skeletal muscles, foetal tissues, placenta, heart, brain, contaminated water and foods and where the infective stages are in low amounts, sensitive procedures are vital to detection of the infection (Ahmad, 2014 and Shapaan, 2015). In blood samples, parasitemia is rarely detected using conventional PCR as stated by Liu et al., (2015). No studies were found that reported finding the parasite DNA in blood of sheep and goats in the country and this was a significant knowledge gap. A number of multicopy targeting genes are used to detect *Toxoplasma gondii* in the samples such as the 529bp multicopy repeat element, the B1 gene, 18S rDNA and the internal transcribed spacer (ITS-1) sequences for optimal sensitivity in addition to single-copy genes such as SAG1 (also known as P-30 antigen), SAG2, and GRA1 (Liu *et al.*, 2015). The 529 bp repeat element is ten to 100 times more sensitive than the B1 gene, 18S rDNA and the internal transcribed spacer (ITS-1) sequences (Liu *et al.*, 2015).

According to Tilahun, (2015), the conventional PCR is performed by first denaturing the template double stranded DNA to single stranded DNA, specific primers that are short single stranded oligonucleotides then anneal to the single stranded DNA at specific sites where a polymerase enzyme identifies the annealed primers and triggers synthesis of new double stranded DNA as the process is repeated in cycles resulting in an exponential increase in the amount of DNA after each cycle. The products of specific DNA are then electrophoresed using agarose gel stained with ethidium bromide and visualized under illuminations of ultraviolet light (Tilahun, 2015).

The amount of *Toxoplasma gondii* tachyzoites in blood of a host and the amount of non-specific DNA in a test sample determines the detection limit of conventional PCR (Hanafiah *et al.,* 2018). In nested PCR, the amplicons from the first reaction are used as templates in the second reaction thus has a superior sensitivity and specificity compared to conventional PCR (Liu *et al.,* 2015).

Other diagnostic molecular methods include real-time PCR and Loop-Mediated Isothermal Amplification (LAMP) while other molecular technologies such as Random Amplification of Polymorphic DNA (RAPD-PCR), Restriction Fragment Length Polymorphism (PCR-RFLP), multilocus sequence typing, High Resolution Melting (HRM) and microsatellite analysis are useful for genotyping in epidemiological studies (Liu *et al.,* 2015).

The sensitivity and specificity of PCR depends on the DNA extraction protocol of which there is no standard, and optimization of conditions during the reaction (Tilahun, 2015). The use of PCR may be limited by being extremely liable to contamination, expensive equipment and reagents, random distribution of the parasite and differing densities of parasite in affected tissue including the need for highly skilled personnel (Mose *et al.,* 2016). The reaction time for PCR is also prolonged (Lau *et al.,* 2010).

2.10 Prevention and Control

Administration of monesin, decoquinate or sulfamezathine potentiated with pyrimethamine has been found to reduce transplacental transmission and fetal mortality in sheep and other livestock (Gebremedhin, 2014). Vaccination of sheep with the S48 live vaccine (Toxovax®) has been found to be more effective as a preventive measure for a minimum period of 18 months in the United Kingdom, France and New Zealand (Dubey, 2009). Reducing the number of cats and other felids around farm premises and avoiding contamination of animal feeds and water with cats' faeces is an important control measure (Dubey, 2009). In humans, spiramycin, atovaquone

or pyremethamine combined with sulfadoxine, have been used to treat the immunocompromised individuals but are not very effective due to inadequate concentrations penetrating bradyzoites (Daka, 2014). Avoiding contact with potential infection sources such as cats, soil, raw meat and unpasteurized milk are primary methods of control (Daka, 2014). Other preventive measures include control of disease vectors such as rodents, flies and cockroaches, cleaning utensils often, washing hands after contact with cat litter and treatment of mothers identified with acute *Toxoplasma* infection as well as education of at-risk groups are vital in the control of toxoplasmosis (Daka, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Kajiado County in the southern part of the republic of Kenya that is bordered by Nairobi and Machakos counties northwards, Narok and Kiambu counties westwards, Makueni and Taita-Taveta counties eastwards and the republic of Tanzania southwards (GoK, 2013). The County has five Sub-Counties; Kajiado North, Kajiado South, Kajiado East, Kajiado West and Kajiado Central. These sub-counties are further sub-divided into a total of 25 wards. The County covers an area of approximately 21,900.9km^2 and lies between longitudes 36º 5' and 37º 5' East and latitude 1º 0' and 3º 0' South (GoK, 2013). It has a human population of 687,312 that mainly comprises of the Maasai community while the estimated livestock population comprises of 411,840 cattle, 718,950 sheep and 699,658 goats (Kenya National Bureau of Statistics (KNBS) 2009).

The main economic activity is livestock rearing engaging about 70% of the human population and other economic activities include agriculture, tourism and other commercial exploits. In addition, the County is among the arid and semi-arid lands (ASALs) in Kenya (GoK, 2013). It experiences a bimodal rainfall with short rains from October to December and long rains from March to May averaging between 300-1,250mm annually (GoK, 2013). Kajiado County has a cool dry climate with mean annual temperatures in most areas being around 21°C (MoALF, 2017). Temperatures vary with altitude and season with the highest temperatures of about 34°C while the lowest is of about 10°C (GoK, 2018). Drought is the main agricultural hazard in the county, commonly resulting in a reduction in the availability of pasture and water (MoALF, 2017). Based on livestock concentrations and convenience, Kaputiei North and Kenyawa-Poka Wards (in Kajiado East Sub-County), Ildamat and Matapato South Wards (in Kajiado central

Sub-County) and Iloodokilani, a Ward in Kajiado West Sub-County, were surveyed for this study.

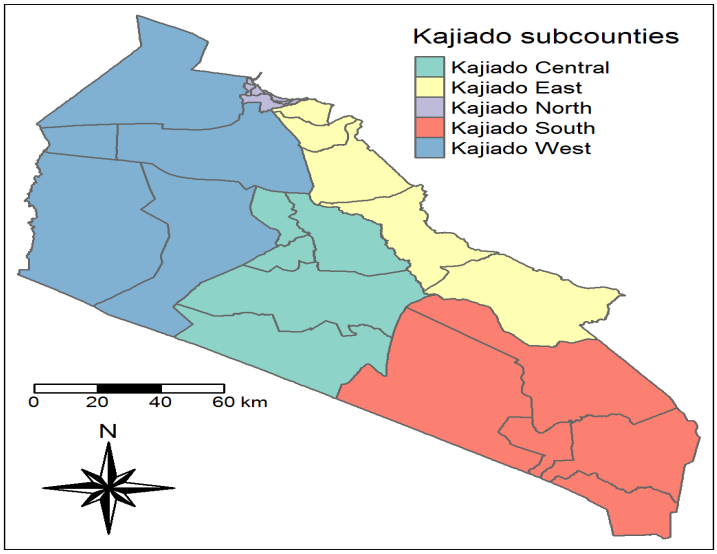

Figure 2: Kajiado county map showing sub-counties and wards (Source: BBSRC-NRF Project Protocol and Guidelines, March 2019**).**

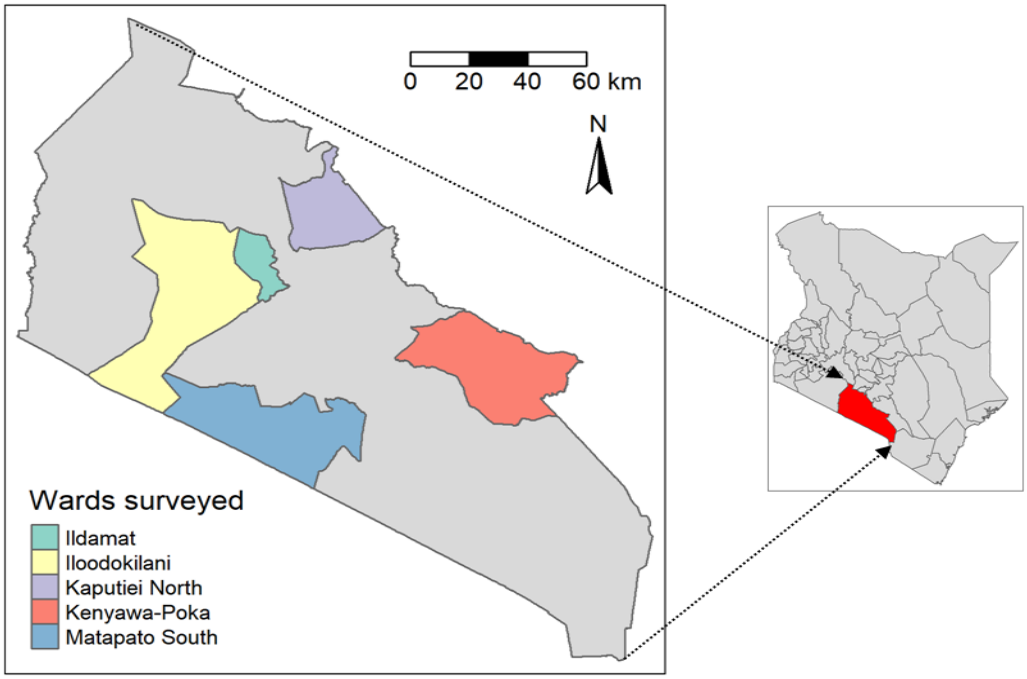

Figure 3: Kajiado county map highlighting wards surveyed (Courtesy: Dr. Christina Ballesteros)**.**

3.2 Study Design

The study design was cross-sectional involving a retrospective survey where interviews were conducted in addition to personal observation. Thereafter, blood samples were collected from the respondents' sheep and goats.

3.3 Target Population

The study targeted all sheep and goats in Kajiado County.

3.4 Study Population

The study focused on sheep and/or goat keeping pastoralists with more than 10 ewes and/or does and a minimum overall flock size of 50 small ruminants in Kajiado County, Kenya.

3.5 Sampling Design

Convenient sampling of three Sub-Counties and five Wards based on flock densities and accessibility was done. Ildamat and Matapato South Wards in Kajiado Central Sub-County, Kaputiei North and Kenyawa Poka Wards in Kajiado East Sub-County, and Iloodokilani Ward in Kajiado West Sub-County participated in the study. Pastoralist flocks were conveniently selected with flock size being the selection criterion where pastoralists with more than 10 ewes and/or does and a minimum overall flock size of 50 small ruminants participated in the study. The sampling unit was composed of all the animals the household head was in charge of in one or more flocks and included those that were entrusted to him/her and excluded owned animals sent to other farms. Each sampling unit was identified in homesteads, grazing areas and watering points. Only reproductive females were included in the study.

Reproductive females in a flock were identified by the herdsman as those that were at least two years of age and had given birth at least once. The age was further estimated by mouthing the animals whereby at two years of age, the central and the first lateral pairs of incisors are replaced

by permanent incisors. The reproductive females were separated from the rest of the flock into an isolated enclosure where available or a human barrier and their number determined. Purposive sampling of females with a history of reproductive problems such as abortion and stillbirths was initially used if such animals existed in the flock. Thereafter, if the required number had not been achieved or if females with a history of reproductive problems were non-existent in a flock, simple random sampling was used to obtain females for sampling. In order determine which female animal to sample, a numeric digit was assigned to each and was written on the body using a marker or hand spray then random numbers were obtained using a scientific calculator (Casio® FX 82MS) based on their total number.

3.6 Sample Size

The study was carried out as part of an on-going project hence the project sampling protocol was adopted. The sample size was calculated using the sample size formula for estimating a sample proportion with a desired precision by Dohoo *et al.,* (2009):

$$n = \frac{Z_{\alpha}^{2}\, p\, q}{L^{2}}$$

Where: n = the sample size

p = expected estimate of the prevalence

q = 1-p

Z = Z score for a given confidence level (95%)

L = precision of estimate/allowable margin of error

At 95% confidence level, 10% precision and estimated prevalence of 50%, this gave a sample size of 96 flocks. Adjustment for clustering was done since the sampling was done in animals within a flock using the formula by Dohoo, *et al.,* (2009):

$n' = n\,(1+p\,(m-1))$

Where n' = new sample size,

n = original sample size estimate (96)

p = intra-cluster correlation coefficient (0.03)

m = number of animals sampled per flock (12)

This gave a design effect of 1.33 and a sample size of 127.68. The final sample size was then approximated to 130 households. Therefore, 130 pastoralists were interviewed and their respective flocks sampled.

The sample size formula given by Dohoo, *et al.,* (2009) for detecting disease from a finite population was used to obtain the number of animals (12) that were sampled from each flock:

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

Where: n = the sample size required

α = 1-confidence interval (0.05)

D = estimated minimum number of diseased animals in the flock (flock size x minimum expected prevalence of 20%) (50 x 0.2 = 10)

N = population size (Minimum flock size was 50 animals)

n =11.77 ~ 12

The number of animals sampled from each of the 130 flocks were 12 reproductive ewes and/or does. A total of 1,560 reproductive ewes and/or does were sampled in the study area.

3.7 Data Collection

Data were obtained from a total of 130 flocks: 25 flocks from Matapato South and 27 flocks from Ildamat Wards situated in Kajiado Central Sub-County, 21 flocks from Kenyawa-Poka and 31 flocks from Kaputiei North Wards in Kajiado East Sub-County and 26 flocks from

Iloodokilani Ward in Kajiado West Sub-County. The interviews were conducted within a three-month period.

An interview with the household head or any other mature household member if the head of the household was unavailable was conducted in swahili or the local maasai language with the aid of a translator from the Directorate of Veterinary Services in the County.

A questionnaire in Open Data Kit (ODK) program (https://opendatakit.org/), a mobile data collection tool, was used to assist in obtaining information on events within the households in the last 12 months (Appendix 1). The following information was obtained: gender, age, level of education and civil status of the pastoralist; size of the flocks owned; feeding of flocks; breeds of sheep and goats; ownership of cats, dogs and cattle; source of water during dry and rainy season; movement of animals for pastures and water; history of abortion, stillbirths and neonatal mortality; consumption of raw meat and raw milk by pastoralists; use of gloves when handling abortion material and knowledge of zoonotic diseases. Pre-testing of the questionnaire was done where seven pastoralists randomly picked participated but were not part of the final analysis of the study. This assisted in determining the accuracy of the questionnaire in capturing the required information, its suitability and time required to complete it. Personal observation was also used to obtain data on household settings, production system used, presence of wild felids, animals being led out early to feed on acacia tree pods that had fallen to the ground at night and more were shaken from the trees, free ranging goats grazing and scavenging in garbage dumps in the outskirts of Kajiado town, and consumption of raw milk by children as they milked the sheep and goats. The breeds of sheep and goats reared by the pastoralists were recognized phenotypically by use of predominant coat colour as described by König *et al.,* (2016) since no breeding records were available.

One flock of each pastoralist participating in the study was used for biological sampling of 12 female animals (six ewes and six does, or all ewes or all does or unproportioned number of females from either species depending on the flock structure). Global positioning system (GPS) coordinates of each flock location were also recorded during the time of sampling.

3.8 Specific Procedures

3.8.1 Collection of blood samples

A total of 1,560 sheep and goats (ewes = 896; does = 664) were sampled for 4-5ml of whole blood and 4-5ml of blood for sera extraction during the study period. Blood was obtained from the jugular vein of the sheep and goats using a sterile venipuncture needle (18-20G), a plastic needle holder and a plain red-topped vacutainer tube for blood for sera extraction and ethylenediamine tetra-acetic acid (EDTA) coated purple-topped vacutainer tube for whole blood. Restraint was done by standing astride the shoulders of the animal and positioning the head upwards. The site was cleaned with 70% ethanol. The jugular vein was raised by pressing the thumb into the jugular furrow at the base of the neck. The needle was pushed sharply through the skin at an angle of 30° into the vein. The vacutainer tube was pushed onto the venipuncture needle for blood to fill the tube. Pressure on the jugular furrow was withdrawn prior to removal of the needle. The vacutainer tubes were labeled with waterproof markers, placed immediately into a cool box with ice packs, then transported to the laboratory at the University of Nairobi, Department of Public Health, Pharmacology and Toxicology.

The whole blood was stored immediately in a deep freezer at -20°C pending DNA extraction. The blood for serum extraction was allowed to stand for at least two hours to allow the clot to develop. Aliquots of sera were obtained by decanting into serum tubes. The sera were also stored immediately in a deep freezer at -20°C pending ELISA screening test.

3.8.2 Indirect ELISA

The sera of 1,464 animals from 122 flocks were tested for anti-*Toxoplasma gondii* IgG antibodies using indirect ELISA multispecies diagnostic kit (ID VET Innovative Diagnostic, ID Screen®, France) while following manufacturer's instructions with limited modifications. This kit had *Toxoplasma gondii* P30 (SAG1) as coated antigen and anti- IgG multi-species horseradish peroxidase (HRP) conjugate (10X) as an antibody-binding reagent. Only the required number of sera was thawed per working day. A sample record sheet with twelve columns (1-12) and eight rows (A-H) was used for ease of tracking the test samples using a laboratory number and for manual recording of results.

To dilute the serum, 90µl of dilution buffer 2 was added to each of the antigen coated micro-wells of the 96-well plate. 10µl of each serum sample to be tested was added to 92 wells leaving out the last four wells (E12- H12) for controls. Positive controls and negative controls were included in each run where 10µl of the negative control (NC) was added to each of E12 and F12 wells while 10µl of the positive control (PC) was added to G12 and H12 wells as indicated in the figure 4 below:

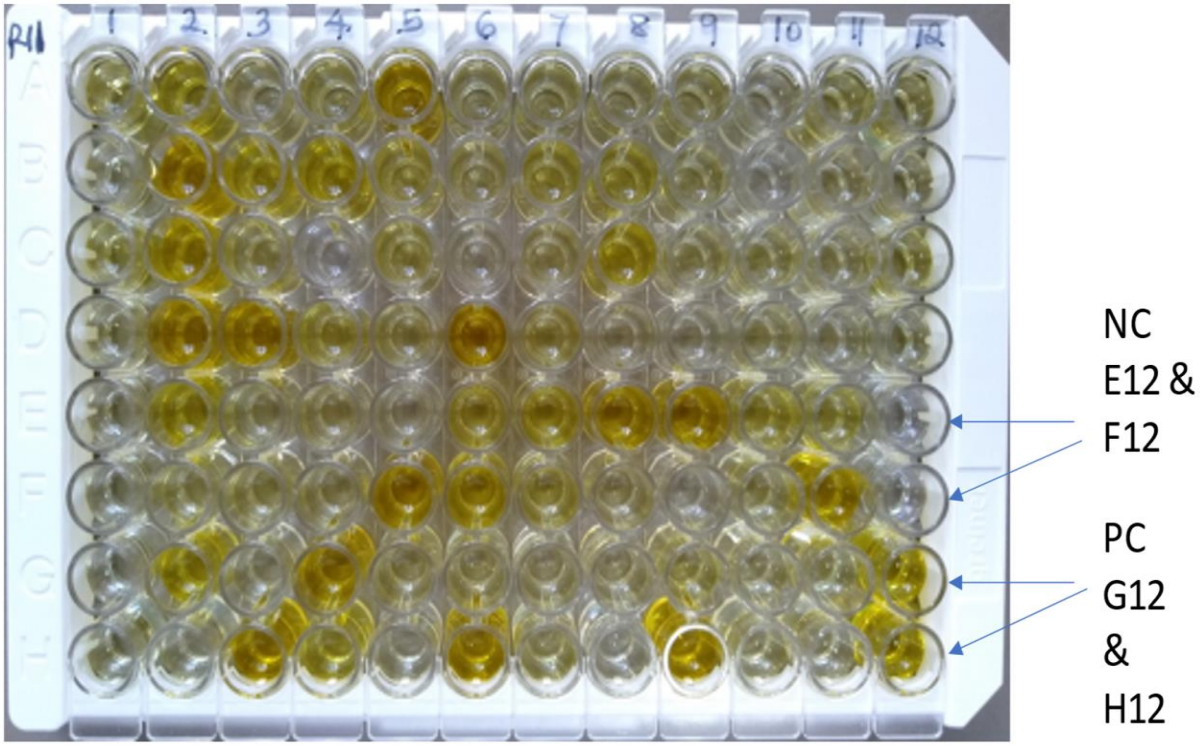

Figure 4: An ELISA plate in use showing the positive control (PC) and negative control (NC) wells.

The manufacturer had recommended use of wells A1 and B1 for negative controls and wells C1 and D1 for positive controls. This was modified to avoid chances of the positive controls spilling over into the test samples while washing the plate manually.

The contents of the plate were then incubated at room temperature (21ºC ± 5ºC) for 45 minutes (± 4 minutes). The 60ml wash concentrate (20X) was brought to room temperature and mixed thoroughly by gentle shaking so as to be completely solubilized. To prepare the wash solution, 1140ml of distilled water were added into a clean container using a measuring cylinder and 60ml of the wash concentrate was added to make a total volume of 1200ml of wash solution and thereby diluting the wash concentrate to 1:20 in distilled water. After the 45 minutes of incubation, the wells were emptied by swiftly inverting the plate at 180º into a sink. The plate was then tilted at about 45º over the sink and the wash solution was added ad lib from a strawed

pressure bottle into each well starting from column 12 to column 1 to avoid the positive control spilling over into the test samples. The plate was agitated by hand for a few seconds then emptied into the sink. The plate was then slightly dried using paper towels placed on a bench by gently tapping the inverted plate. The washing was done three times to eliminate unbound material while avoiding complete drying of the wells between washings.

To prepare the conjugate (1x), that contains an enzyme-linked secondary antibody which bind to the preceding antibody, 1ml of concentrated conjugate (10x) was added to 10ml of dilution buffer 3 thereby diluting the concentrated conjugate (10x) to 1/10. 100µl of conjugate (1x) was then added to each well and the plate incubated for 30 minutes (±3 minutes) at room temperature of 21ºC (± 5ºC). The wells were then emptied into a sink and washing of the wells was done as described earlier.

After drying, 100µl of the substrate solution was then added to each well and then incubated for 15 minutes (± 2 minutes) at 21ºC (± 5ºC) in the dark, in this case, the plates were placed into an empty drawer in the laboratory. After the 15-minute incubation, positive reactions showed a blue colour if antigen-antibody reaction occurred. The reaction was stopped by adding 100µl of the stop solution to each well. A yellow colour was produced whose optical density (O.D) was read within 15 minutes of stopping the reaction at 450nm using a microplate spectrophotometer (Multiskan ex®, Thermo Electron Corporation). The spectrophotometer 450nm filter size was obtained by selecting 'MEAS PARAM', then 'FILTER NO' (number 2) and pressing 'ENTER'.

The tests were validated by calculating the mean values of the two positive controls O.D (ODPC) where if greater than 0.350 (ODPC > 0.350), the tests were valid.

For each sample, the sample to positive percentage (S/P%) was calculated using a computer spreadsheet (Microsoft® Excel 2010) using the following the following formula as per the manufacturers' instructions:

$$S/P\% = \frac{OD\ sample - ODNC}{ODPC - ODNC} \times 100$$

Animals whose serum samples presented an S/P% greater than or equal to 50% were considered positive, those with greater than 40% but less than 50% were considered doubtful while those less than or equal to 40% were considered negative. A flock was considered as *Toxoplasma gondii* seropositive when at least one animal from the flock tested positive.

3.8.3 Extraction of *Toxoplasma gondii* DNA

The genomic DNA of *Toxoplasma gondii* was extracted from thawed whole blood of sheep and goats using a commercial kit (Thermo Scientific® GeneJET Whole Blood Genomic DNA Purification Mini Kit) following manufacturers' instructions with no modification as follows:

The wash buffers were prepared by adding 120ml of 100% ethanol to each of the 40ml bottles of concentrated wash buffer I (WB I) and wash buffer II (WB II) to make a diluted volume of 160ml of each buffer. Thereafter the check box on the bottle caps were marked to indicate the addition of ethanol as instructed. Proteinase K and the required amount of blood samples for DNA extraction per day were thawed before use while the lysis solution was checked for precipitation before use. A set of Eppendorf tubes (1.5ml), corresponding to each blood sample, were prepared by labelling with waterproof markers. Then 200µl of whole blood sample pipetted into the tubes. Thereafter, 20µl of proteinase K was added into each tube and mixed by vortexing for 10 seconds. Then, 400µl of lysis solution was added to the mixture in each tube and vortexed for 15 seconds. The tubes were capped tight and secured onto a rack with sticky tape and then

incubated at 56°C in a water bath for 10 minutes to digest proteins. Thereafter, 200 µL of 100% ethanol was added to the mixture in each tube and vortexed for 15 seconds. The mixture was then transferred into correspondingly labelled spin columns inserted into collection tubes. The column cap was closed and the mixture centrifuged at 8,000 rpm for 1 minute in order to bind the DNA to the column. The flow-through solution in the collection tube was discarded and the DNA bound to the column was retained. Thereafter, 500µl of diluted wash buffer (WB I) was added into the column and the column cap was closed tightly and centrifuged at 10,000 rpm for 1 minute. The flow-through solution was discarded and the column with bound DNA retained in the collection tube. Once more, 500 µl of diluted wash buffer (WB II) was added into the column, the cap closed and then centrifuged at 15,000 rpm for 3 minutes. The collection tubes were emptied off the flow-through solution and the columns retained in the tubes. A dry spin of the columns lasting one minute at 15000rpm was then carried out. The columns were then transferred into correspondingly labelled sterile 1.5ml Eppendorf tubes. To elute the bound DNA in the column, 200 µl of elution buffer was added into the column and the mixture allowed to incubate for 2 minutes at room temperature. The columns were then centrifuged at 10,000 rpm for 1 minute and the eluted DNA recovered in the 1.5 ml Eppendorf tubes. Thereafter, the eluted DNA samples were then stored at -20°C pending analysis by conventional Polymerase Chain Reaction (PCR).

3.8.4 Amplification of *Toxoplasma gondii* DNA by PCR

The study utilized conventional PCR to detect presence of *Toxoplasma gondii* DNA in the blood of 154 sheep and goats that were seropositive. This was also analyzed in an additional 92 DNA samples from eight flocks of animals that had not been screened with ELISA due to a faulty kit. This was to ensure Toxoplasma positivity was determined in all the 130 flocks that were sampled

in the study area. The genomic DNA of *Toxoplasma gondii* was detected using specific primers designed to targeted the 420bp multicopy element, a genetic marker of the organism as described by Kong *et al.,* (2012). To identify the organism, the specific nucleotide primers that were used to amplify the specific genes of the organism were a forward F_o -mcrr 5'-TGACTCGGGCCCAGCTGCGT-3' and a reverse R_o -mcrr 5'-CTCCTCCCTTCGTCCAAGCCTCC-3' targeting 420bp of the multicopy repeat region (Acc. No. AF146527.1) of *Toxoplasma gondii* (Kong *et al.,* 2012).

The amplifications were done in a 25µl mixture containing 0.6µl (10µM) of each primer (Macrogen®, Netherlands), 12.5µl of Gotaq® G2 Hot Start Green Master-Mix 2X (Promega® corporation) (containing 0.1 U Taq Polymerase, 500 µM dNTP each, 20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3 mM $MgCl_2$), 3µl of DNA template, and 8.3µl of nuclease-free water.

The 96-well thermocycler (Bio-Rad® T100) was used for amplification of the genes. Initial denaturation was done by heating the PCR mixture to 94°C for five minutes then denaturation at 94°C for 10 seconds in order to separate the double stranded DNA. The annealing of the forward and reverse primers was at 60°C for 15 seconds. The temperature was then increased to 72°C for 20 seconds to allow for new DNA synthesis (extension). These denaturation, annealing and extension processes were repeated for 30 cycles. A final extension at 72°C for seven minutes concluded the process to enable the DNA polymerase to finalize the DNA synthesis at optimal temperature. To examine the quality and the banding intensity of the amplicons, they were separated by gel electrophoresis in 1.5% agarose gel pre-stained with 14µl of ethidium bromide and 3µl of 100bp DNA ladder was used as a molecular size marker. The electrophoresed and stained DNA was visualized with a UV transilluminator (Gelmax® 125 Imager, UVP,

Cambridge, UK) and photographs of the stained DNA in gel were taken using the gel viewer. An amplicon size of 420bp was obtained.

3.8.5 Purification of PCR amplicons for sequencing

Eighteen of the *Toxoplasma gondii* strongly positive PCR products were purified using a commercial kit (GeneJET Gel Extraction Kit, Thermo scientific®, USA) where the gel containing the DNA fragment was excised over a UV illuminator (Vilber Lourmat®) using a clean scalpel as close as possible to the DNA band to minimize the gel volume. The gel was placed into a pre-weighed 1.5ml Eppendorf tube, weighed and the weight recorded. For every 100mg of 2% agarose gel, 200µl of binding buffer was added. The gel mixture was then incubated at 60ºC in a water-bath for 10 minutes and inverted every few minutes until the gel slice was completely dissolved while checking maintenance of yellow colour that indicated optimal pH for DNA binding. Then 100µl of 100% isopropyl alcohol was added to every 100mg of agarose gel and mixed thoroughly. Up to 800µl of the solubilized gel solution was then transferred to the purification column and centrifuged for a minute. The flow-through solution was discarded and the column placed back into the same collection tube. Where the total volume exceeded 800µl, the solution was added to the column in stages, centrifuged and the flow-through solution discarded until the entire volume was processed. An additional binding step was taken by adding 100µl of binding buffer to the purification column, centrifuged for a minute and the flow-through solution discarded but the collection tube was retained. The column was then washed by adding 700µl of wash buffer diluted with ethanol, centrifuged for a minute and the flow-through solution discarded but the collection tube retained. The empty purification column was centrifuged for an additional minute to completely remove the buffer since ethanol may inhibit downstream enzymatic reactions. The column was transferred into a clean 1.5ml microcentrifuge

tube, 30µl of elution buffer was then added to the center of the column membrane and centrifuged for a minute. The column was discarded and the purified DNA stored at -20ºC. Thereafter, the purified PCR amplicons were packed and sent to Macrogen® (Amsterdam, Netherlands) for sequencing.

3.8.6 Analysis of DNA sequences

The sequenced DNA were then analysed using the basic local alignment search tool (BLAST) of NCBI GenBank database. The query sequences were compared with those in the database in order to identify the *Toxoplasma gondii* organism. A 98-100% similarity to homologues found in the GenBank database was compared with the sequenced DNA during BLAST analysis.

3.9 Data Analysis

Data collected from the respondents using a questionnaire were entered into a computer spread sheet (Microsoft Excel®2010) for cleaning and coding followed by descriptive analysis in the statistical package for social sciences (IBM SPSS® version 25.0). Data from quantitative variables were expressed as the mean, median, standard deviation and range while qualitative variables were estimated and expressed as frequencies and percentages and their corresponding 95% confidence intervals determined. The level of significance was set at $P<0.05$ for purposes of statistical interpretation. The data from the questionnaire was then matched with the corresponding laboratory results and were analyzed for potential risk factors using generalized linear modelling. A forward approach was utilized where variables that had a p-value < 0.05 in the univariate analysis were used in the development of the multivariate analysis model. These variables are listed in supplementary table (Appendix 2). Prevalence

was determined as the percentage of number of positive animals divided by the number of tested animals. The independent samples t-test was performed using Instat+® version 3.36.

3.10 Ethical Consideration

Ethical approval for conducting the study was obtained from the University of Nairobi Faculty of Veterinary Medicine Biosafety, Animal Use and Ethics Committee (FVM BAUEC/2020/255) (Appendix 3). Approval to conduct the study in the study area was granted from the Directorate of Veterinary Services, Kabete, through the County Director of Veterinary Services in Kajiado County (Appendix 4). Furthermore, the objectives and details of the study written in a consent form were explained to the pastoralists by two local animal health assistants who participated in the study using the local maasai language. The pastoralists were then asked if they were willing to participate in the study and those who accepted were requested to sign the consent forms (Appendix 5). Therefore, informed written consent was obtained before the interviews were conducted.

CHAPTER FOUR

4.0 RESULTS

4.1 Description of demographic information

In this study, a total of 130 pastoralist household heads were interviewed where 114 (87.7%) were males and 16 (12.3%) were females. Majority (64/130, 49.2%) were above 51 years of age, 52 (40%) were between 36-50 years old whereas 14 (10.8%) were below 35 years of age. Most of them had formal education where 31 (23.8%) had gone up to primary level, 41 (31.5%) up to secondary level and 17 (13.1%) up to college or university level of education. Nearly a third (41/130, 31.5%) of the pastoralists had no formal education but it was observed that most could communicate in swahili language. Most of the pastoralists (124/130, 95.4%) were married, 5 (3.8%) were widows or widowers and only one was single. Most pastoralists (127/130, 97.7%) were living as single nuclear family units in modern housing made of iron sheet roofing and earthen, wooden or stone walls and only three (2.3%) lived in a '*manyatta',* the traditional household setting among the Maasai community where several families or a clan live together under one headship. This survey revealed that most pastoralists kept both sheep and goats. This study revealed that most of the pastoralists (94.6%; 123/130) kept sheep and goats mainly for regular income, five (3.8%) kept them as a source of meat and milk and only two revealed that they reared them for prestige purposes. Other than sheep and goats, 118 (90.8%) pastoralists also owned cattle, 127 (97.7%) also owned dogs and 119 (91.5%) also owned cats (Table 1).

Table 1: Description of demographics of the pastoralists keeping small ruminants in Kajiado county.

Variable	**Category**	**N**	**%**
Gender	Males	114	87.7
	Females	16	12.3
Age of respondents (years)	< 35 years	14	10.8
	36-50 years	52	40.0
	≥ 51 years	64	49.2
Level of education	Primary	31	23.8
	Secondary	41	31.5
	College/university	17	13.1
	No formal education	41	31.5
Marital status	Married	124	95.4
	Widow/widower	5	3.8
	Single	1	0.8
Households living in '*manyatta'*	Yes	3	2.3
Households rearing sheep	Yes	129	99.2
Households rearing goats	Yes	115	88.5
Purpose of keeping sheep and goats	Regular cash income	123	94.6
	Source of meat and milk	5	3.8
	Prestige	2	1.5
Households rearing cattle	Yes	118	90.8
Households owning cat	Yes	119	91.5
Households owning dog	Yes	127	97.7

Key: *'manyatta'* is the traditional household setting among the Maasai community where several families or a clan live together under one headship.

The highest number of sheep owned at the ward level were in Iloodokilani ward with a mean of 78.81, a median of 55, a minimum of 21 and a maximum of 287 sheep in a flock while Matapato south had the lowest with a mean of 46.08, a median of 40, a minimum of seven and a maximum of 110 sheep in a flock. The highest number of goats owned at ward level were in Kenyawa Poka ward with a mean of 61.38, a median of 42, a minimum of zero and a maximum of 200 goats in a flock while Kaputiei north had the lowest with a mean of 36.65, a median of 25, a minimum of zero and a maximum of 170 goats in a flock (Table 2).

Table 2: Descriptive statistics on number of small ruminants owned by pastoralists in Kajiado county.

	Number of sheep owned				Number of goats owned			
Ward	**Mean**	**Median**	**Min.**	**Max.**	**Mean**	**Median**	**Min.**	**Max.**
Ildamat	63.56	48	0	300	41.89	30	0	200
Matapato south	46.08	40	7	110	53.68	36	16	241
Iloodokilani	78.81	55	21	287	43	35.5	0	150
Kenyawa poka	59.81	58	22	120	61.38	42	0	200
Kaputiei north	77.16	70	12	230	36.65	25	0	170

Key: Min. = minimum, Max. = maximum

4.2 Breeds of sheep and goats

Nearly all of the 130 pastoralists kept sheep except one whereas only fifteen did not have goats. Sheep were mainly dorper and red maasai crosses (69/129, 53.5%). There were some who apparently owned dorper breed (50/129, 38.8%) and the red maasai breed (10/129, 7.8%). The goat breeds reared were predominantly galla (57/115, 49.6%) and galla and small East African crosses (55/115, 47.8%). The small East African breed was reared by a few of the pastoralists (3/115, 2.6%) (Table 3).

Table 3: Breeds of sheep and goats reared by the pastoralists

Variable	**Category**	**N**	**%**
Breed of sheep	Dorper	50	38.8
	Red maasai	10	7.8
	Crosses (Dorper x red maasai)	69	53.5
Breed of goats	Galla	57	49.6
	Small East African (SEA)	3	2.6
	Crosses (Galla x SEA)	55	47.8

4.3 Production systems used in rearing sheep and goats

In this study, it was observed that most of the flocks (126/130, 96.9%) were kept under extensive system and only four flocks (3.1%) were under semi-intensive system. The latter were located in peri-urban areas near Namanga and Kajiado towns. Majority of the pastoralists (101/130, 77.7%)

fed their sheep and goats by exclusively grazing them on pastures while the rest supplemented them on minerals, concentrates, hay and crop residues. It was also observed that the animals were led out early in the morning to pick the protein-rich acacia tree pods from the ground that had fallen during the windy nights and more were shaken from the trees by the herders. The animals would then proceed to routine grazing. The sheep and goats flocks were observed to be grazed separately from cattle herds. Grazing was mostly on pastoralists' own land (90/130, 69.2%), 21 (16.2%) used communal land while 18 (13.8%) used public land.

This study also revealed that nearly a third (39/130, 30%) had moved their animals away from home in the last 12 months before the study in search of pastures and water. Some went up to Makindu and Chyulu hills in Makueni county to the north, Narok county to the west, Amboseli national park, Namanga hills and into the republic of Tanzania territory to the south. During the rainy season, 55 (42.3%) of the pastoralists reported that they sourced water from waterpans for their animals, 54 (41.5%) from rivers, 9 (6.9%) from their own wells, 8 (6.2%) from communal wells, 3 (2.3%) used piped water and only one (0.8%) used harvested rain water. In the dry season, most pastoralists (75/130,57.7%) sourced water from communal wells, 31 (23.8%) use their own wells, 19 (14.6%) use waterpans, 3 (2.3%) use piped water and only one (0.8%) indicated that he sourced water from the river (Table 4).

Table 4: Production systems used in rearing sheep and goats

Variable	Category	N	%
Production system	Extensive	126	96.9
	Semi-intensive	4	3.1
Feeding	Grazing only	101	77.7
	Grazing, minerals and hay	9	6.9
	Grazing and minerals	10	7.7
	Grazing and concentrates	6	4.6
	Grazing and crop residues	1	0.8
	Grazing and hay	3	2.3
Ownership of grazing land	Pastoralists' own land	90	69.2
	Public land	21	16.2
	Communal land	18	13.8
Movement of animals for pastures and water	Yes	39	30
Source of water during rainy season	Water-pan	55	42.3
	Own well	9	6.9
	Communal well	8	6.2
	River	54	41.5
	Piped water	3	2.3
	Harvested rain water	1	0.8
Source of water during dry season	Water-pan	19	14.6
	Own well	31	23.8
	Communal well	75	57.7
	River	1	0.8
	Piped water	3	2.3

4.4 Disease and reproductive challenges experienced by the pastoralists

The main diseases and conditions reported to affect the sheep and goats were worm infection (62.3%, 81/130) and respiratory diseases (28.5%, 37/130). Furthermore, seventy-four (56.9%) pastoralists reported history of abortion in their animals in the last 12 months. Majority of the pastoralists (50/130, 38.5%) stated infectious diseases as the cause of abortion in their flocks. In addition, forty (30.8%) indicated to have had their animals delivering stillbirths in the last 12 months. In flocks where females carried pregnancy to term, eighty-seven (66.9%) pastoralists reported neonatal mortality (Table 5).

Table 5: Disease and reproductive challenges experienced by the pastoralists

Variable	Category	N	%
Main diseases and conditions reported in sheep and goats	Helminthiasis	81	62.3
	Respiratory diseases	37	28.5
	Other parasites	3	2.3
	Foot and mouth disease	2	1.5
	Tick infestation	2	1.5
	Diarrhoea	1	0.8
	Footrot	1	0.8
	Orf	1	0.8
	Peste des petit ruminantes	1	0.8
	Skin disease	1	0.8
History of abortion in sheep and goats	Yes	74	56.9
Reasons for abortion	Infectious disease	50	38.5
	Plant poisoning	13	10
	Vaccination	3	2.3
	Brucellosis	2	1.5
	Rift valley fever	3	2.3
	Enterotoxaemia	1	0.8
	Tick infestation	1	0.8
	Serving pregnant animals	1	0.8
	Twinning	1	0.8
	Injury	1	0.8
History of stillbirths in sheep and goats	Yes	40	30.8
History of neonatal mortality in lambs and kids	Yes	87	66.9

The number of abortions reported had a mean of 1.68 and 2.69 in ewes and does respectively with a minimum of one and a maximum of five in ewes and a minimum of one and a maximum of 12 in does. The number of stillborn reported had a mean of 1.57 and 1.77 in lambs and kids respectively with a minimum of one and a maximum of five in both species. The number of dead lambs and kids had a mean of 3.39 and 3.93 respectively with a minimum of one and a maximum of 15 lambs and a minimum of one and a maximum of 18 kids per flock during the study period (Table 6).

Table 6: Descriptive statistics on reproductive challenges reported by pastoralists in sheep and goats in Kajiado county during the study period.

Variable	Mean	SD	Minimum -maximum
Number of abortions in ewes	1.68	1.055	1-5
Number of abortions in does	2.69	2.573	1-12
Number of stillborn lambs	1.57	0.898	1-5
Number of stillborn kids	1.77	1.110	1-5
Number of lambs dead	3.39	2.822	1-15
Number of kids dead	3.93	3.371	1-18

Key: SD = standard deviation

4.5 Determination of prevalence of Toxoplasma gondii in sheep and goats in Kajiado county using ELISA.

Sera from 1,464 adult female sheep and goats was tested with indirect ELISA where 10.5% (154/1464;95% CI: 9.0-12.2) of the animals tested positive. The prevalence of *Toxoplasma gondii* was 9.0% (76/842; 95% CI: 7.3-11.2) and 12.5% (78/622; 95% CI: 10.2-15.4) in sheep and goats respectively. This was statistically significantly higher in goats than in sheep (P = 0.0337). Sixty-six flocks of sheep and goats had at least one positive animal, therefore, the overall prevalence was determined to be 54% (66/122; 95% CI:45.3-62.7) at flock level. Kenyawa poka ward had the highest prevalence (15.9%), followed by Matapato south (14.72%), Ildamat (12.5%), Kaputiei north (9.1%) while Iloodokilani had the lowest at 2.57% (Table 7).

Table 7: Prevalence of *Toxoplasma gondii* in small ruminants in Kajiado county using ELISA

Location (wards)	Sample size (n=1,464)	Positive cases	%
Ildamat	264	33	12.5
Matapato south	265	39	14.72
Iloodokilani	311	8	2.57
Kenyawa poka	252	40	15.9
Kaputiei north	372	34	9.1

An independent samples t-test was used to check for differences in infection with *Toxoplasma gondii* in the two animal species in the five wards. This revealed that there was a statistically

significant difference in infection between sheep and goats in Ildamat ward (t (263) = 3.51, *P* = 0.0004). No significant differences were found in Matapato south (t (264) = 0.85, *P* = 0.3954), Iloodokilani (t (310) = 0.19, *P* = 0.8469), Kenyawa poka (t (251) = 0.71, P = 0.4775) and Kaputiei north wards (t (371) = 1.94, p = 0.0527). The difference in Kaputiei North was almost statistically significant (Table 8).

Table 8: Number of sheep and goats sampled and the number testing positive for *Toxoplasma gondii* using ELISA.

Ward	**Sheep**	**Goats**	**t**	***P* value**
Ildamat	150 (9)	114 (24)	3.51	0.0004
Matapato south	126 (21)	139 (18)	0.85	0.3954
Iloodokilani	166 (4)	145 (4)	0.19	0.8469
Kenyawa poka	132 (23)	120 (17)	0.71	0.4775
Kaputiei north	268 (19)	104 (15)	1.94	0.0527

Key: The numbers in parenthesis are those that tested positive.

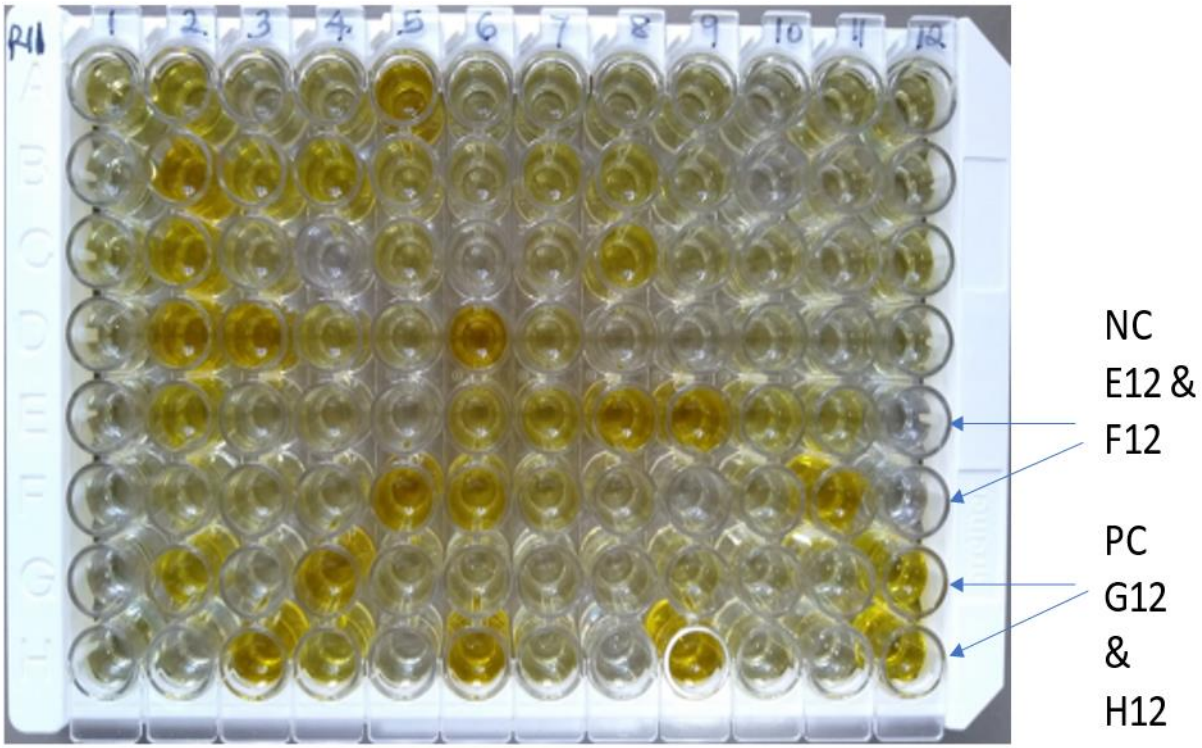

Figure 5: Indirect ELISA plate; E12 and F12 show negative control while G12 and H12 show positive control.

4.6 Detection of *Toxoplasma gondii* DNA in blood of small ruminants by use of PCR in Kajiado county

Conventional PCR was used to sequentially test for the presence of *Toxoplasma gondii* DNA in blood of 154 seropositive sheep and goats. The presence of a specific DNA band corresponding to 420bp was detected in 88.96% (137/154; 95% CI: 83.0-93.0) of these animals thus confirming the presence of *Toxoplasma gondii* DNA. These translated into 88.2% (67/76; 95% CI: 79.0-93.6) prevalence in seropositive sheep and 89.7% (70/78; 95% CI: 81.0-94.7) prevalence in seropositive goats whose difference was not statistically significant (t = 0.31; *P* = 0.7537).

Kaputiei north ward had the highest prevalence (100%) followed by Kenyawa poka (92.5%), Matapato south (92.3%), Iloodokilani (87.5%) while Ildamat ward had the lowest (69.7%) (Table 9).

Table 9: Detection of *Toxoplasma gondii* DNA from blood of small ruminants in Kajiado county using PCR.

Ward	**Number of animals tested (n=154)**	**Positive (%)**
Ildamat	33	23 (69.7)
Matapato south	39	36 (92.3)
Iloodokilani	8	7 (87.5)
Kenyawa poka	40	37 (92.5)
Kaputiei north	34	34 (100)

An independent samples t-test was used to check for differences in infection with *Toxoplasma gondii* in sheep and goats in the five wards. This revealed that there were no statistically significant differences in infection between the two species in any of the wards, that is, Ildamat [t (32) = 0.23, *P* = 0.8194], Matapato South [t (38) = 1.87, *P* = 0.0614], Iloodokilani [t (7) = 1.15, *P* = 0.2482], Kenyawa Poka [t (39) = 0.83, P = 0.4044] and Kaputiei North (Table 10).

Table 10: Number of sheep and goats sampled and the number testing positive for *Toxoplasma gondii* DNA using PCR.

Ward	Sheep	Goats	t	P value
Ildamat	9 (6)	24 (17)	0.23	0.8194
Matapato South	21 (18)	18 (18)	1.87	0.0614
Iloodokilani	4 (3)	4 (4)	1.15	0.2482
Kenyawa Poka	23 (22)	17 (15)	0.83	0.4044
Kaputiei North	19 (19)	15 (15)	-	-

Key: The numbers in parenthesis are those that tested positive.

Other 92 blood samples from about eight flocks that had not been screened using ELISA due to a faulty kit were also tested for *Toxoplasma gondii* DNA out of which 43 were positive that were from each of the eight flocks. Therefore, seventy-four flocks were confirmed to be infected thus *Toxoplasma gondii* positivity was 56.9% (74/130; 95% CI: 48.3-65.1) at flock level.

A comparison of 18 sequenced DNA to homologues in the GenBank database of the NCBI using the basic local alignment search tool (BLAST), revealed that none of the query DNA sequences had a 98-100% BLAST match similarity.

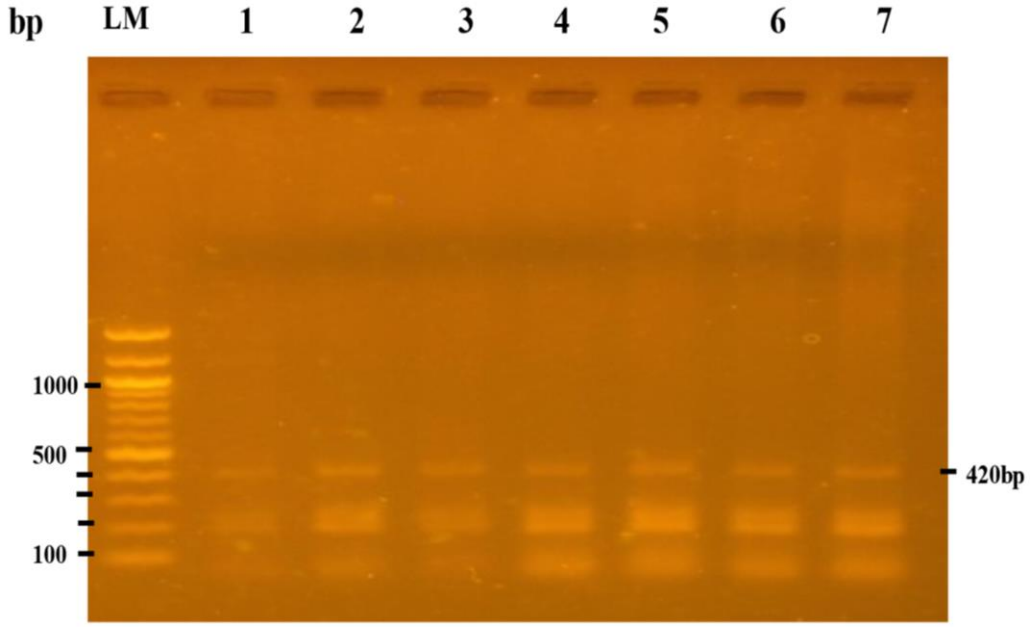

Figure 6: Gel photograph for PCR amplification product showing bands of 420bp of the multicopy element of *Toxoplasma gondii* from blood of infected sheep and goats in Kajiado county. Lane LM:100 bp DNA ladder marker, lanes 1-7 are positive.

4.7 Risk factor analysis for *Toxoplasma gondii* infection in sheep and goats

A univariate analysis was carried out to assess whether there existed an association between *Toxoplasma gondii* positivity in sheep and goats and the various variables. The statistically significant variables with $P < 0.05$ at 95% confidence interval from this analysis were location of animals (Iloodokilani ward (OR 0.637; $P = 0.000$)); feeding by grazing and hay (OR 0.513; $P = 0.032$) and sourcing water from pastoralists' own well during the dry season (OR 1.281; $P = 0.013$). These three variables were then used in multivariate analysis.

The following variables were not significant ($P > 0.05$) in the univariate analysis; breed of sheep, breed of goats, number of flocks, flock sizes, owning cat, dog, or cattle; ownership of land used for grazing, movement of livestock to seek pastures and water, history of stillbirths or abortions, and production system (Table 10). Nonetheless, a higher positivity was observed in pastoralists with two flocks (66.7%) than one flock (57.9%); in small flock sizes (62.9%) than in medium and large flocks; in flocks of red maasai sheep breed (63.6%) than crosses (57.4%) and dorper breed of sheep (54%); in flocks of galla goats (61.4%) than crosses (54.5%) and small East African breed of goats (33.3%); in those flocks with stillbirth (65%) and abortion history (58.1%); in pastoralists' households with cats (57.1%) and dogs (57.5%); in pastoralists' flocks grazing on communal land (66.7%) than public (55.6%) and own land (54.4%); in pastoralists' flocks that did not move (57.1%) than those moved; in pastoralists' flocks under semi-intensive system (75%) than extensive system and in pastoralists' flocks using piped water (100%) and harvested rainwater (100%) than other sources of water during the rainy season (Table 11).

Table 11: Univariate analysis of potential risk factors associated with *Toxoplasma gondii* positivity at flock level.

Variable	N	*n* (*T.gondii* positive)	%(n/N)	*B*	Standard error	OR	*P* value
Location (ward)							
Ildamat	27	17	63	-0.127	0.1248	0.880	0.307
Iloodokilani	25	17	68	-0.451	0.1260	0.637	0.000
Kaputiei North	26	4	15.4	-0.010	0.1209	0.990	0.932
Kenyawa Poka	21	17	81	0.090	0.1331	1.094	0.501
Matapato South	31	19	61.3	Ref.		1	
Household in *manyatta*							
No	127	73	33.3	-0.052	0.2841	0.949	0.853
Yes	3	1	57.5	Ref			
Own sheep							
No	1	1	100	0.388	0.4872	1.473	0.426
Yes	129	73	56.6	Ref			
Own goats							
No	15	8	53.3	-0.017	0.1335	0.983	0.896
Yes	115	66	57.4	Ref			
Number of flocks							
1 flock	121	70	57.9	0.625	0.4788	1.868	0.192
2 flocks	6	4	66.7	0.714	0.5097	2.043	0.161
3 flocks	2	0		-5.995E-15	0.5840	1.000	1.000
4 flocks	1	0		Ref.		1	
Flock size							
Small (1-200)	116	73	62.9	0.201	0.1885	1.222	0.287
Medium (201-300)	7	4	57.1	0.143	0.2589	1.154	0.581
Large (301 and above)	7	3	42.9			ref	
Breed of sheep							
Dorper	50	27	54	0.075	0.1621	1.077	0.646
Cross (Dorper x red maasai)	69	39	56.5	0.072	0.1582	1.075	0.648
Red maasai	10	7	70	Ref			
Breed of goats							
Galla	57	35	61.4	0.316	0.2862	1.371	0.270
Cross (Galla x SEAG)	55	30	54.5	0.267	0.2865	1.306	0.352
Small East African goat (SEAG)	3	1	33.3	Ref			
Stillbirth experience							
No	90	48	53.3	-0.086	0.0921	0.917	0.350
Yes	40	26	65	Ref			
Number of stillborn lambs				-0.012	0.0544	0.989	0.832
Number of stillborn				0.096	0.0526	1.101	0.068

kids							
Abortion experience							
No	56	31	55.4	0.017	0.0862	1.017	0.845
Yes	74	43	58.1	Ref			
Number of abortions in sheep				0.006	0.0393	1.006	0.878
Number of abortions in goats				-0.018	0.0237	0.982	0.440
Own cattle							
No	12	7	58.3	0.056	0.1473	1.058	0.701
Yes	118	67	56.8	Ref			
Own cat							
No	11	6	54.5	-0.076	0.1532	0.926	0.618
Yes	119	68	57.1	Ref			
Own dog							
No	3	1	33.3	-0.289	0.2831	0.749	0.308
Yes	127	73	57.5	Ref			
Neonatal mortality							
No	43	25	58.1	0.053	0.0906	1.055	0.555
Yes	87	49	56.3	Ref			
Number of lambs dead				-0.028	0.0153	0.972	0.065
Number of kids dead				-0.004	0.0146	0.996	0.808
Pasture land ownership							
Own	91	50	54.9	-0.062	0.1254	0.940	0.619
Communal	21	14	66.7	-0.048	0.1561	0.953	0.760
Public	18	10	55.6	Ref			
Feeding of sheep and goats							
Grazing only	101	58	57.4	-0.043	0.1620	0.958	0.791
Grazing and minerals	10	5	50	-0.167	0.2140	0.846	0.436
Grazing and concentrates	6	5	83.3	0.333	0.2455	1.396	0.175
Grazing and crop residues	1	0		-0.667	0.4910	0.513	0.175
Grazing and hay	3	0		-0.667	0.3105	0.513	0.032
Grazing, minerals and hay	9	6	66.7	Ref.			
Movement of livestock							
No	91	52	57.1	-0.037	0.0931	0.964	0.694
Yes	39	22	56.4	Ref			
Production system							
Extensive	126	71	56.3	-0.139	0.2468	0.870	0.574
Semi-intensive	4	3	75	Ref			
Source of water during rainy season							

Harvested rain water	1	1	100	0.500	0.5031	1.649	0.320
Water-pan	55	30	54.5	0.064	0.1795	1.066	0.723
Own well	9	7	77.8	0.389	0.2305	1.475	0.092
River	54	30	55.6	0.111	0.1797	1.118	0.536
Piped water	3	3	100	0.500	0.3211	1.649	0.119
Communal well	8	3	37.5	Ref			
Source of water during dry season							
Water-pan	19	10	52.6	0.105	0.1207	1.111	0.383
Own well	31	23	74.2	0.248	0.1003	1.281	0.013
River	1	1	100	0.474	0.4737	1.606	0.317
Piped water	3	3	100	0.474	0.2770	1.606	0.087
Communal well	75	37	49.3	Ref.			

Key: N = Total number of flocks, n = proportion of infected flocks, OR = odds ratio, Ref. = reference variable.

The multivariate analysis revealed that none of the studied risk factors for occurrence of *Toxoplasma gondii* positivity in sheep and goats were significant ($P > 0.05$) (Table 12).

Table 12: Multivariate analysis of potential risk factors associated with *Toxoplasma gondii* positivity at flock level

Variable	**B**	**Std. error**	**OR**	***P* value**
Ward				
Ildamat	-0.049	0.1273	0.952	0.700
Iloodokilani	-0.252	0.1384	0.778	0.069
Kenyawa Poka	0.215	0.1368	1.240	0.116
Kaputiei North	0.158	0.1425	1.171	0.267
Matapato South	Ref.		1	
Feeding of sheep and goats				
Grazing only	0.112	0.1728	1.118	0.519
Grazing and minerals	-0.028	0.2125	0.972	0.895
Grazing and concentrates	0.379	0.2622	1.461	0.148
Grazing and crop residues	-0.222	0.4708	0.801	0.638
Grazing and hay	-0.398	0.2993	0.672	0.184
Grazing, minerals and hay	Ref		1	
Source of water during dry season				
Piped water	0.389	0.2719	1.475	0.153
Own well	0.119	0.1069	1.126	0.267
Water pan	-0.013	0.1278	0.987	0.919
River	0.464	0.4397	1.591	0.291
Communal well	Ref.		1	

Key: *OR = odds ratio, Ref. = reference variable.*

4.8 Analysis of potential exposure factors for *Toxoplasma gondii* infection in humans

In this study, seventy-four pastoralists (74/130, 56.9%) were found to have flocks of sheep and goats that were *Toxoplasma gondii* positive. Sixty-eight (57.1%) of them owned cats. Sixty-three (60%) of those who assist their own animals when they abort had infected flocks. Sixty-seven (56.8%) of those who do not use gloves while assisting animals during abortion or when handling abortion material had infected flocks. In addition, 71(56.8%) of those who somewhat dispose the abortion materials by giving to pets had infected flocks. Notably, one pastoralist who revealed that he gives the abortion materials to pets or uses it for human consumption had an infected flock. Notably, fifty-one (54.3%) pastoralists who did not know of any zoonotic disease had infected flocks. The zoonotic diseases that the pastoralists knew about were anthrax, brucellosis, rift valley fever, and rabies. None of the pastoralists cited toxoplasmosis as a zoonotic disease.

In addition, this study revealed that 15 (57.7%) of the pastoralists who consumed raw meat occasionally had *Toxoplasma gondii* infected flocks. On the other hand, 34 (52.3%) of the pastoralists who consumed raw blood either always or on occassion had infected flocks. Four (50%) of the pastoralists who reported to have occasionally consumed raw sheep or goat milk had infected flocks. In addition, it was observed that children drank raw milk as they milked the household flocks of sheep and goats. Furthermore, 24 (55.8%) pastoralists with infected flocks always or occasionally fermented raw sheep or goat milk for consumption. In spite of these practices, over half of the pastoralists (53.3%) who did not know that humans could acquire disease from milk had infected flocks. Nevertheless, thirty-seven pastoralists reported they knew brucellosis as a disease acquired from milk while others stated they knew of tuberculosis, diarrhoea, brucellosis and rift valley fever. Forty-nine (54.4%) of those who did not know of any specific disease acquired from milk had infected flocks (Table 13).

Table 13: Analysis of potential exposure factors for human infection with *Toxoplasma gondii*.

Risk factor	**Categories**	**(n/130) (%)**	***T.gondii* positive (n)**	***T.gondii* positive (%)**
Presence of *Toxoplasma gondii* in flock	Yes	74 (56.9)	-	-
Household owning cat	Yes	119 (91.5)	68	57.1
Assistance during abortion	Self	105 (80.8)	63	60
	Veterinarian	4 (3.1)	4	100
	None	20 (15.4)	6	30
	Others	1 (0.8)	1	100
Use of gloves during abortion	Always	5 (3.8)	4	80
	Never	118 (90.8)	67	56.8
	Occasionally	7 (5.4)	3	42.9
Disposal of abortion material	Pet	100 (76.9)	55	55
	Latrine	4 (3.1)	3	75
	Burying	1 (0.8)	0	0
	Pet and bury	5 (3.9)	3	60
	Pet and latrine	19 (14.6)	12	63.2
	Pet and human consumption	1 (0.8)	1	100
Knowledge of zoonotic disease	No	93 (71.5)	51	54.8
	Yes	37 (28.5)	23	62.2
Knowledge of specific zoonotic disease	Anthrax	10 (7.7)	8	80
	Brucellosis	15 (11.5)	7	46.7
	Rabies	2 (1.5)	2	100
	Rift valley fever	6 (4.6)	2	33.3
		1 (0.8)	1	100
	Many zoonoses	96 (73.8)	54	56.3
	No known disease	0		
	Toxoplasmosis			
Consumption of raw meat	Always	1 (0.8)	0	0
	Never	103 (79.2)	59	57.3
	Occasionally	26 (20.0)	15	57.7
Consumption of raw blood	Always	2 (1.5)	2	100
	Never	65 (50.0)	40	61.5
	Occasionally	63 (48.5)	32	50.8
Consumption of raw milk	Always	1 (0.8)	0	0
	Never	121 (93.1)	70	57.9
	Occasionally	8 (6.2)	4	50
Use of raw milk to make ‘*maziwa mala’* (fermented milk)	Always	23 (17.7)	12	52.2
	Never	87 (66.9)	50	57.5
	Occasionally	20 (15.4)	12	60
Knowledge of disease from milk	No	90 (69.2)	48	53.3
	Yes	40 (30.8)	26	65

CHAPTER FIVE

5.0 DISCUSSION

This study reported the prevalence of *Toxoplasma gondii* infection in sheep and goats in Kajiado County. This was determined by use of indirect ELISA and confirmed using conventional PCR. The seroprevalence of this parasite was 9.0% (76/842) in sheep and 12.5% (78/622) in goats. *Toxoplasma gondii* DNA was detected in 89% (137/154) of the seropositive animals by use of conventional PCR.

In this study, the 9% prevalence determined in sheep was comparable to 8.3% prevalence reported by Dahmani *et al.,* (2018) in Algeria and 11.8% by Dong *et al.,* (2018) in China using ELISA. However, it was lower than 56% prevalence earlier reported by Abwajo, (1984) in Kenya using indirect ELISA. The higher prevalence in the previous study in the country could be attributed to random sampling from slaughterhouses as well as differences in validation of the serological tests used. The prevalence was also lower than 33.7% reported by Tilahun *et al.,* (2018) in Ethiopia and 45.7% reported by Atail *et al.,* (2017) in Sudan where indirect ELISA was used. It was also noticeably lower than 26.1% estimated in Africa by Tonouhewa *et al.,* (2017) and the 30% global prevalence estimated by Samra *et al.,* (2007). This can be attributed to differences in geographical location and animal husbandry practices.

In this study, the 12.5% prevalence of *Toxoplasma gondii* determined in goats was comparable to 15% global prevalence estimated by Samra *et al.,* (2007). It also compares to 14.32% reported by Ahmad *et al.,* (2015) in Pakistan, 16.3% reported by Amairia *et al.,* (2016) in Tunisia and 17.6% estimated by Dong *et al.,* (2018) in China using ELISA. It also closely compares to 15.48% prevalence reported by Gebremedhin *et al.,* (2014) in Ethiopia using Direct Agglutination Test (DAT). Nevertheless, the prevalence in goats was apparently lower than 21% reported earlier by Abwajo (1984) in Kenya using ELISA and 19.3% reported by Swai and Kaaya, (2012) in

Tanzania using Latex Agglutination Test (LAT). It was also lower than reported in other parts of Africa for instance 27.6% reported by Tilahun *et al.,* (2018) in Ethiopia, 27.2% reported by Atail *et al.,* (2017) in Sudan, 31% reported by Bisson *et al.,* (2000) in Uganda, 34.5% reported by Lahmar *et al.,* (2015) in Tunisia and 41.7% reported by Ghoneim *et al.,* (2010) in Egypt where ELISA was used. It was also lower than 22.9% prevalence estimated by Tonouhewa *et al.,* (2017) in Africa.

The overall 10.5% prevalence of *Toxoplasma gondii* in the current study is lower than that reported in some African countries and globally. This differs from earlier studies carried out in Ghana, Pakistan, Brazil and China that reported a higher prevalence in sheep and goats reared under extensive system (Tonouhewa *et al.,* 2017). The probability of ingesting *Toxoplasma gondii* oocysts contaminated pastures and water is higher under an extensive system than under semi-intensive and intensive systems (Tonouhewa *et al.,* 2017). The presence of domestic cats near households was reported by respondents in this study while wild felids were observed in the study area (personal observation). These are the definitive hosts of *Toxoplasma gondii* likely to contaminate the environment with faeces containing its oocysts if infected. Therefore, the low prevalence determined in this study can be attributed to dry climatic conditions in the study area which is a characteristic arid and semi-arid area, that is inimical to the survival of *Toxoplasma gondii* oocysts in the environment as described by Gebremedhin *et al.,* (2014) in a study in East Shewa zone, Ethiopia. Oocysts lose their ability to sporulate under extreme heat or solar radiation and once sporulated, they survive best in moist soil and moderate temperatures (Shapiro *et al.,* 2019). Dry climatic conditions have been incriminated to lower the prevalence of *Toxoplasma gondii* in sheep under extensive system in South Africa (Samra *et al.,* 2007). A dry climate is a common phenomenon in areas where a similar low prevalence has been reported in

Africa such as Algeria (Dahmani *et al.*, 2018), Tunisia (Amairia *et al.*, 2016) and some parts of Ethiopia (Gebremedhin *et al.,* 2014). Livestock in the study area are reared in extensive grasslands under a pastoralist system though land use is changing with time as observed by Nyariki *et al.*, (2009). Nevertheless, low prevalence can also be attributed to low antibody levels during early infection that may lead to low sensitivity with indirect ELISA (Lau *et al.*, 2010).

In this study, the seroprevalence was observed to be statistically significantly higher in goats than in sheep ($P = 0.0337$). Therefore, the findings contrast with most of other researchers who reported a higher prevalence of toxoplasmosis in sheep compared to goats. For instance, Gebremedhin *et al.,* (2014) reported 20% and 15% prevalence in sheep and goats respectively in Ethiopia using DAT and attributed the higher prevalence in sheep to feeding habits whereby sheep are grazers and more likely to be infected from oocysts contaminated pastures than goats who are browsers. The higher prevalence in goats in the current study could also be attributed to feeding habits. The animals were observed being led out early in the morning to feed on acacia tree pods that had fallen during the night and more would be shaken from the smaller trees by the herdsmen (personal observation). Goats are voracious towards tree pods since they are browsers and could have been consuming more of the pods than sheep and making them more exposed to infection since the pods fell on bare ground where they were likely to be contaminated with oocysts from infected domestic and wild felids as suggested by Shapiro *et al.,* (2019). Contaminated outdoor water sources have been recognized to be a high risk for toxoplasmosis infection in goats (Ahmad *et al.,* 2015). However, source of water was not found to be a significant risk factor for infection in sheep and goats in this study. This study therefore recommends further studies to clarify on this difference. Ahmed *et al.,* (2016) and Gharekhani *et al.,* (2018) have also reported a higher prevalence in goats than sheep in Pakistan and Iran

respectively. Ahmed *et al.,* (2016) attributed this to the higher activity and movement in goats than sheep.

In this study, Ildamat ward was observed to have had a statistically significant difference in the numbers of seropositive goats than sheep (t (263) = 3.51, P = 0.0004). This probably indicated that the goats in this ward were more exposed to *Toxoplasma gondii* infection than sheep. Ildamat ward hosts Kajiado town where free ranging goats were observed grazing and scavenging in garbage dumps in the outskirts of the town (personal observation). Some of those free ranging goats participated in the study. The pastures around the town are likely to be contaminated with infected cat faeces from litter boxes or faeces from stray and wild cats that are likely to scavenge in the same environment for food.

In this study, *Toxoplasma gondii* DNA was detected in the blood of 88.96% (137/154) of the seropositive sheep and goats by use of conventional PCR. However, 11% (17/154) of the seropositive animals were negative. In blood samples, parasitemia is rarely detected using conventional PCR as stated by Liu et al., (2015). The findings from this study are therefore phenomenal. In order to verify the sequence of the PCR products,18 of the PCR positive samples were sequenced by BLAST analysis in the NCBI database revealed that none of the query DNA sequences had a 98-100% BLAST match similarity. This failure to match the sequences could be attributed to low concentration of DNA or copurification of amplification inhibitors or other factors as suggested by Kircher and Kelso (2010) since the target 420bp bands were clearly visible in the agarose gel after electrophoresis. No other study has established molecular prevalence of *Toxoplasma gondii* in sheep and goats in Kenya making it difficult to compare findings from other researchers in the country. Toxoplasmosis is one of the neglected zoonotic diseases in the country as reported by Mbabu *et al.,* (2014) and Munyua *et al.,* (2016).

The PCR technique is highly sensitive in detecting antigens. The amount of *Toxoplasma gondii* tachyzoites in blood of a host and the amount of non-specific DNA in a test sample determines the detection limit of conventional PCR (Hanafiah *et al.,* 2018). *Toxoplasma gondii* tachyzoites are only detectable in blood during acute infection since they form tissue cysts in various organs in chronic infection (Gebremedhin, 2014). In this study therefore, the 88.96% of seropositive animals that tested positive with PCR could have been suffering from an acute infection while the 11% of the seropositive animals that were PCR negative demonstrated previous exposure to the parasite. Acute infection in the animals in this study can be supported by reports of abortion (56.9%), stillbirths (30.8%) and neonatal mortality (66.9%) experienced by the pastoralists in the last 12 months before the study. These reproductive problems can be influenced by disease among other factors as cited by Nyariki *et al.,* (2009). They are the principal clinical reproductive problems associated with *Toxoplasma gondii* infection in animals (Ahmad, 2014 and Atail *et al.,* 2017). It was noteworthy that infectious diseases were cited as probable causes of abortion by 38.5% of the pastoralists. Gebremedhin *et al.,* (2013) and Cenci-Goga *et al.,* (2013) associated *Toxoplasma gondii* infection with abortion in a study in Ethiopia and Italy respectively. The infection has been confirmed as a cause of abortion in Europe, USA and New Zealand (Dubey, 2009). The common zoonotic protozoal diseases that cause reproductive problems in man and animals include toxoplasmosis, sarcosporidiosis, neosporosis and trypanosomiasis where toxoplasmosis is most significant (Shaapan, 2015). Other infectious diseases associated with reproductive problems in sheep and goats include brucellosis, leptospirosis, *Chlamydia* and Q fever infection (OIE, 2012a).

In this study, there were no statistically significant flock level risk factors to *Toxoplasma* positivity in sheep and goats. The univariate analysis showed that location of animals in

Iloodokilani ward (OR 0.637; $P = 0.000$)); feeding by grazing and hay (OR 0.513; $P = 0.032$) and sourcing of water from pastoralists' own well during the dry season (OR 1.281; $P = 0.013$) were significantly associated with *Toxoplasma* positivity ($P < 0.05$). However, these variables were found not to be independent predictors of positivity from the multivariate analysis ($P > 0.05$). The strength of association indicated by the odds ratio was weak in these variables. This is a knowledge gap where further studies are necessary.

This study also analyzed potential risk factors for human infection. It can be extrapolated that the seropositive animals pose a risk to human infection if their meat, blood or milk were consumed raw by the pastoralists or if bare hands are used to handle their meat or abortion material. The study revealed that seventy-four pastoralists (56.9%) had flocks of sheep and goats that were *Toxoplasma gondii* positive and these could serve as source of infection to humans as described by Gebremedhin *et al.,* (2014). It is likely that these flocks acquired infection from wild felids (personal observation) since no correlation was found between cat ownership and flock infection in this study. The life cycle of *Toxoplasma gondii* may involve wild felids as stated by Rouatbi *et al.,* (2019). They share the same environment with the flocks and therefore, infected ones can contaminate pastures and watering points with faeces containing *Toxoplasma gondii* oocysts.

This study showed that 90.8% of the pastoralists do not use gloves while assisting the animal or when handling abortion material 56.8% of them had *Toxoplasma gondii* positive animals. This finding compares to that of Ogendi *et al.,* (2013) who reported that none of the farmers in Thika region of Kenya used gloves to handle abortion material. It has been reported that handling infected aborted matter or contact with foetal fluids without protective gear is a probable source of exposure to zoonoses (Onono *et al.,* 2019). Moreover, over a hundred of the pastoralists gave

aborted matter to pets (dogs and cats) and over half of them had *Toxoplasma gondii* positive flocks. This can result into development of characteristic oocysts of *Toxoplasma gondii* in their intestines as reported by Gebremedhin *et al.,* (2015) and eventual contamination of the environment with oocysts in cat faeces. The development of oocysts in dogs is uncertain and is an area of further study. One pastoralist revealed that he also used aborted matter for human consumption and he had an infected flock. This was noted to be a risky practice if viable cysts were not destroyed during preparation.

This study also revealed that majority of the pastoralists (71.5%) did not know of any zoonotic disease and over half of them had infected animals. In addition, none among those who knew some zoonotic diseases mentioned toxoplasmosis. In comparison, Ogendi *et al.,* (2013) reported that only one individual in their study knew about toxoplasmosis suggesting that the disease is largely unknown in the country.

This study found that 26 pastoralists had occasionally consumed raw meat during the study period and over half of them had infected animals. This habit could contribute to their infection since goat meat, mutton or other meat products containing *Toxoplasma gondii* tissue cysts have been reported to be the main sources of human infection in Africa (Tenter (2009) and Tonouhewa *et al.,* (2017)). Previously, it had been observed that consumption of raw meat is one of the inherent cultural practices among pastoralist communities in Kenya (Onono *et al.,* 2019). Animal parts commonly eaten raw include the kidneys, the liver and the fatty tails of sheep (Chege *et al.,* 2015). This practice has also been reported in other African countries for instance, eating undercooked grilled mutton and goat meat is common in Egypt (Al Kappany *et al.,* 2018). In contrast, non-pastoralist livestock owners usually consume properly cooked meat as reported by Ogendi *et al.,* (2013).

In this study, two pastoralists reported to have always consumed raw blood during the study period while 63 others consumed it occasionally where over half of them had infected animals. Tachyzoites of *Toxoplasma gondii* circulate in blood of acutely infected animals and consumption of their raw blood poses a risk to infection. Mixing of raw blood with raw milk and traditional herbs is a common practice among the Maasai pastoral settings and the drink is also encouraged for children and infants (Chege *et al.,* 2015). This poses a risk of infection among the young as well. In addition, eight pastoralists reported to have occasionally consumed raw sheep or goat milk and this study revealed that half of them had *Toxoplasma gondii* infected animals. Children took raw milk as they milked the animals (personal observation). Other studies have revealed that pastoralists encourage children to take raw milk in an attempt to promote their immunity against infections (Onono *et al.,* 2019). It is ostensive that their attempt to have better health can be counter-productive.

Fermented raw sheep or goat milk was also reported to be frequently or occasionally consumed by 43 of the pastoralists in this study, over half of whom had infected animals. Dubey *et al.,* (2014) detected *Toxoplasma gondii* in fresh goat cheese indicating that the parasite can survive in fermented milk products. Raw sheep and goat milk can be a source of human infection since it has been found to be contaminated with *Toxoplasma gondii* tachyzoites (Saad *et al.,* 2018 and Shapiro *et al.,* 2019). Muhie and Keskes (2014) reported that unpasteurized milk from acutely diseased goats is a source of *Toxoplasma gondii* infection to humans. Tenter (2009) concluded that any type of raw milk is a potential source of human infection. Contrary to findings in this study, Ogendi *et al.,* (2013) reported that consumption of raw milk is not a common practice among non-pastoralist communities in a survey of farmers in Thika region of Kenya on risk

factors for *Toxoplasma gondii* infection in humans where nearly all respondents preferred boiled milk.

In this study, it was established that most of the pastoralists (69.2%) did not know that humans could acquire diseases from milk. Furthermore, toxoplasmosis was not among the diseases alluded to be acquired from raw milk and this indicated that it is not known in this pastoral community. This inference is reinforced by the finding that 31.5% of the participants in this study had no formal education and only 13.1% had college or university level of education. Recent studies among the pastoralist communities in the study area have also reported low awareness of diseases among non-educated populations (Onono *et al.*, 2019). Low education levels or being illiterate have been found to be a risk factor for zoonotic infections in humans as it diminishes the understanding of hygiene principles (Paul *et al.,* 2018). However, deep-rooted cultural practices and beliefs may hinder change of behavior even among the educated.

Limitations in this study

This study was limited by several factors:

1. Sampling of flocks was challenged by poor accessibility due to a rough terrain in the study area.
2. This study only sampled female animals therefore had no opportunity to compare differences in *Toxoplasma gondii* infection with the sex of the animals.
3. Ninety-two serum samples from eight flocks were not screened using ELISA due to a faulty kit.
4. A positive control was not available for use in the PCR analysis.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions were made from this study:

1. *Toxoplasma gondii* is present in Kajiado county with a seroprevalence rate of 9% in sheep and 12.5% in goats. *Toxoplasma gondii* DNA was detected in 88.96% of the seropositive animals. The prevalence of *Toxoplasma gondii* implies that toxoplasmosis is likely to be one of the contributing factors to reproductive problems in sheep and goats in the study area.
2. The prevalence of *Toxoplasma gondii* was significantly higher in goats than sheep. This difference was postulated to be due to the feeding habits of the animals.
3. The potential exposure factors to human infection with *Toxoplasma gondii* in Kajiado County included failure to use gloves while handling aborted materials from livestock, consumption of raw blood, raw meat and unpasteurized milk; as well as pastoralists' lack of knowledge on zoonotic diseases. This implies that there is likelihood of significant human infection in the study area.

6.2 Recommendations

1. This study recommends the need for public health education as a way of reducing transmission of *Toxoplasma gondii* in both animals and man and therefore reducing its prevalence and potential impact on human and animal health.
2. Although the study examined various risk factors to infection with *Toxoplasma gondii* in sheep and goats, no association was identified in the study area. Further study is therefore recommended.

3. This study also recommends further studies to clarify the reason for higher prevalence of *Toxoplasma gondii* in goats than sheep in the study area.
4. It is requisite for further studies be conducted in order to determine the prevalence and risk factors for *Toxoplasma gondii* infection in other food animals such as cattle, pigs and camels and among the pastoralists and other communities in Kenya.
5. This study also recommends more research on neglected zoonotic diseases in the country as part of One-Health approach.

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APPENDICES

Appendix 1: Questionnaire/observational assessment for risk factors

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

Name of interviewer: ……..……..

Respondents' personal information

Name of respondent (optional) ……..…….....

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

Age (years): ……...

Gender (Tick appropriate box): ☐ Male ☐ Female ☐ Other

Level of education:

- ☐ None
- ☐ Primary school
- ☐ Secondary school
- ☐ College/university
- ☐ Other

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

Living in a '*manyatta'* (observational): ☐ Yes ☐ No

Questions regarding animals in the farm:

1. Do you have cats around the homestead? ☐ Yes ☐ No
2. Do you own dogs? ☐ Yes ☐ No
3. Do you own cattle? ☐ Yes ☐ No

4. What is your main reason for keeping sheep and goats?
   - ☐ Regular cash income
   - ☐ Source of meat and milk
   - ☐ Prestige
   - ☐ Other reasons

5. Number of livestock kept:
   (i) Total number of sheep: …..……..(Number will be 0 if don't have)
   (ii) Total number of goats…….. (Number will be 0 if don't have)
   (iii) Number of flocks of sheep and goats……..……. ……
   (iv) Number of cattle …….. (Number will be 0 if don't have)

6. Breeds of sheep (observational):
   - ☐ Red maasai
   - ☐ Dorper

☐ Somali
☐ Other breeds (Specify)……
☐ I don't have

7. Breed of goats: (observational)
☐ Galla
☐ Small east African
☐ Alpine
☐ Other breeds (Specify)……
☐ I don't have

8. Type of management system for sheep and goats (observational):
☐ Intensive
☐ Semi-intensive
☐ Extensive

9. How do you feed your sheep and goats?
☐ Grazing only
☐ Grazing, minerals and hay
☐ Grazing and minerals
☐ Grazing and concentrates
☐ Grazing and crop residues
☐ Grazing and hay

10. Who owns the land where you graze your sheep and goats?
☐ Own land
☐ Communal land
☐ Public land

11. Did you move your flocks to seek pastures and water in the last 12 months? ☐ Yes ☐ No
12. a) Have you experienced abortion in your flocks in the last 12 months? ☐ Yes ☐ No
b) If yes, number of abortions in:
(i) Sheep……...
(ii) Goats……..

c) Who assisted your ewes and does when they were aborting?
☐ Self
☐ Veterinarian
☐ Others
☐ No assistance

d) How do you dispose the abortion material?
☐ Giving to pet
☐ Throwing into Latrine
☐ Burying
☐ Human consumption

☐ Other method……. (specify)

13. How often do you wear gloves when handling aborted material?
    - ☐ Always
    - ☐ Occasionally
    - ☐ Never

14. Please indicate the main cause of abortion in your sheep and goats:
    - ☐ Infectious disease
    - ☐ Plant poisoning
    - ☐ Vaccination
    - ☐ Brucellosis
    - ☐ Rift valley fever
    - ☐ Enterotoxaemia
    - ☐ Tick infestation
    - ☐ Serving pregnant animals
    - ☐ Twinning
    - ☐ Injury
    - ☐ Other……. (specify)

15. a) Have you experienced stillbirths in your flocks in the last 12 months? ☐ Yes ☐ No
    b) If yes, number of stillbirths in:
        (i) Sheep……
        (ii) Goats……..

16. a) Have you experienced deaths of lambs and kids in your flocks in the last 12 months?
    ☐ Yes ☐ No

    b) If yes, number of lambs and kids dead:
        (i) Lambs……..
        (ii) Kids……..

17. What is the main disease or condition affecting your sheep and goats?
    - ☐ Worm infection
    - ☐ Respiratory diseases
    - ☐ Foot and mouth disease
    - ☐ Tick infestation
    - ☐ Diarrhoea
    - ☐ Foot rot
    - ☐ Orf
    - ☐ Peste des ruminantes
    - ☐ Skin disease
    - ☐ Other……..(specify)

18. Sources of water for sheep and goats (during last rainy season):
    - ☐ Piped water

- ☐ River/stream
- ☐ Wells/ boreholes
- ☐ Water pan
- ☐ Roof harvested rainfall
- ☐ Other……....

19. Sources of water for sheep and goats (during last dry season):
    - ☐ Piped water
    - ☐ River/stream
    - ☐ Wells/ boreholes
    - ☐ Water pan
    - ☐ Roof harvested rainfall
    - ☐ Other……....

Risk eating habits in humans:

20. Do you slaughter sheep and goats at home for consumption?
    ☐ Yes ☐ No

21. How often do you drink raw blood from sheep or goat?
    - ☐ Always
    - ☐ Occasionally
    - ☐ Never

22. How often do you eat raw sheep/goat meat?
    - ☐ Always
    - ☐ Occasionally
    - ☐ Never

23. How often do you drink raw sheep/goat milk?
    - ☐ Always
    - ☐ Occasionally
    - ☐ Never

24. How often do you make fermented milk (‘*maziwa mala’*) from raw milk?
    - ☐ Always
    - ☐ Occasionally
    - ☐ Never

Disease knowledge

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

b) If yes, mention the disease.
- ☐ Anthrax
- ☐ Brucellosis
- ☐ Rabies
- ☐ Rift valley fever

☐ I don't know
☐ Other……..… (specify)

26. a) Are you aware of any disease, which can be acquired through consumption of milk from sheep and goats to human? ☐ Yes ☐ No

b) If yes, mention the disease

☐ Brucellosis
☐ Rift valley fever
☐ Diarrhoea
☐ Tuberculosis
☐ I don't know
☐ Other……(specify)

Appendix 2: Supplementary tables (Risk factor analysis)

i. Univariate analysis

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	Df	Sig.		Lower	Upper
(Intercept)	.720	.0900	.544	.896	64.063	1	.000	2.054	1.722	2.451
[ward=Ildamat]	-.127	.1248	-.372	.117	1.042	1	.307	.880	.689	1.124
[ward=Iloodoki]	-.451	.1260	-.698	-.204	12.801	1	.000	.637	.498	.816
[ward=Kapu_Nor]	-.010	.1209	-.247	.227	.007	1	.932	.990	.781	1.254
[ward=Ke_Poka]	.090	.1331	-.171	.350	.452	1	.501	1.094	.842	1.420
[ward=Mata_Sou]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.202[b]	.0251	.159	.258						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.667	.2808	.116	1.217	5.635	1	.018	1.948	1.123	3.377
[mnytt=no]	-.052	.2841	-.609	.504	.034	1	.853	.949	.544	1.656
[mnytt=yes]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.237[b]	.0293	.186	.302						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	Df	Sig.		Lower	Upper
(Intercept)	.612	.0427	.529	.696	205.400	1	.000	1.845	1.697	2.006
[Own_sheep=0]	.388	.4872	-.567	1.342	.633	1	.426	1.473	.567	3.829
[Own_sheep=1]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.236[b]	.0292	.185	.300						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.617	.0454	.528	.706	185.226	1	.000	1.854	1.696	2.026
[Own_goat=0]	-.017	.1335	-.279	.244	.017	1	.896	.983	.756	1.277
[Own_goat=1]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.237[b]	.0294	.186	.302						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	5.884E-15	.4768	-.935	.935	.000	1	1.000	1.000	.393	2.546
[no_flk=1]	.625	.4788	-.313	1.563	1.704	1	.192	1.868	.731	4.775
[no_flk=2]	.714	.5097	-.285	1.713	1.964	1	.161	2.043	.752	5.547
[no_flk=3]	-5.995E-15	.5840	-1.145	1.145	.000	1	1.000	1.000	.318	3.141
[no_flk=4]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.227[b]	.0282	.178	.290						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	Df	Sig.		Lower	Upper
(Intercept)	.429	.1830	.070	.787	5.482	1	.019	1.535	1.072	2.198
[Flock size=0]	.201	.1885	-.169	.570	1.134	1	.287	1.222	.845	1.769
[Flock size =1]	.143	.2589	-.364	.650	.305	1	.581	1.154	.695	1.916
[Flock size =2]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.235[b]	.0291	.184	.299						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	Df	Sig.		Lower	Upper
(Intercept)	.545	.1468	.258	.833	13.812	1	.000	1.725	1.294	2.300
[Breed_sheep=cross]	.072	.1582	-.238	.382	.208	1	.648	1.075	.788	1.466
[Breed_sheep=dorper]	.075	.1621	-.243	.392	.211	1	.646	1.077	.784	1.480
[Breed_sheep=red_m]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.237[b]	.0295	.186	.302						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.333	.2790	-.213	.880	1.428	1	.232	1.396	.808	2.411
[breed_goat=cross]	.267	.2865	-.295	.828	.866	1	.352	1.306	.745	2.289
[breed_goat=galla]	.316	.2862	-.245	.877	1.217	1	.270	1.371	.783	2.403
[breed_goat=sea]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.233[b]	.0308	.180	.302						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.675	.0767	.525	.825	77.518	1	.000	1.964	1.690	2.282
[stborn:exp=no]	-.086	.0921	-.267	.094	.873	1	.350	.917	.766	1.099
[stborn:exp=yes]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.235[b]	.0292	.184	.300						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.620	.0470	.527	.712	173.957	1	.000	1.858	1.695	2.037
No_stborn_lam	-.012	.0544	-.118	.095	.045	1	.832	.989	.889	1.100
(Scale)	.237[a]	.0293	.186	.302						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.587	.0450	.498	.675	169.949	1	.000	1.798	1.646	1.964
No_:stborn_kid	.096	.0526	-.007	.199	3.329	1	.068	1.101	.993	1.220
(Scale)	.231[a]	.0286	.181	.294						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.608	.0565	.497	.719	115.651	1	.000	1.837	1.644	2.052
[abrt:exp=no]	.017	.0862	-.152	.186	.038	1	.845	1.017	.859	1.204
[abrt:exp=yes]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.237[b]	.0293	.186	.302						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	Df	Sig.		Lower	Upper
(Intercept)	.611	.0516	.510	.712	140.147	1	.000	1.842	1.665	2.038
abort_sheep	.006	.0393	-.071	.083	.024	1	.878	1.006	.931	1.087
(Scale)	.237[a]	.0294	.186	.302						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.629	.0461	.539	.719	186.273	1	.000	1.876	1.714	2.053
abort_goat	-.018	.0237	-.065	.028	.597	1	.440	.982	.937	1.028
(Scale)	.236[a]	.0292	.185	.300						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.610	.0448	.522	.698	185.824	1	.000	1.841	1.686	2.010
[own_cattle=no]	.056	.1473	-.232	.345	.147	1	.701	1.058	.793	1.412
[own_cattle=yes]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.236[b]	.0293	.185	.301						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.622	.0446	.535	.709	194.793	1	.000	1.862	1.707	2.032
[own_cat=no]	-.076	.1532	-.377	.224	.249	1	.618	.926	.686	1.251
[own_cat=yes]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.236[b]	.0293	.185	.301						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square	df	Sig.	Exp(B)	95% Wald Confidence Interval for Exp(B) Lower	Upper
(Intercept)	.622	.0430	.538	.706	209.285	1	.000	1.863	1.712	2.027
[own_dog=no]	-.289	.2831	-.843	.266	1.040	1	.308	.749	.430	1.305
[own_dog=yes]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.235[b]	.0291	.184	.299						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square	df	Sig.	Exp(B)	95% Wald Confidence Interval for Exp(B) Lower	Upper
(Intercept)	.598	.0521	.496	.700	131.667	1	.000	1.818	1.641	2.013
[neo_mort:exp=no]	.053	.0906	-.124	.231	.348	1	.555	1.055	.883	1.260
[neo_mort:exp=yes]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.236[b]	.0293	.185	.301						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square	df	Sig.	Exp(B)	95% Wald Confidence Interval for Exp(B) Lower	Upper
(Intercept)	.674	.0529	.571	.778	162.702	1	.000	1.963	1.770	2.177
lamb:lam_dead	-.028	.0153	-.058	.002	3.404	1	.065	.972	.943	1.002
(Scale)	.231[a]	.0286	.181	.294						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.621	.0491	.525	.718	160.041	1	.000	1.861	1.691	2.049
kid_dead	-.004	.0146	-.032	.025	.059	1	.808	.996	.968	1.025
(Scale)	.237[a]	.0293	.186	.302						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.667	.1146	.442	.891	33.865	1	.000	1.948	1.556	2.438
land=com	-.048	.1561	-.354	.258	.093	1	.760	.953	.702	1.295
land=own	-.062	.1254	-.308	.183	.247	1	.619	.940	.735	1.201
land=pub	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.236[b]	.0293	.185	.301						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.641	.0779	.488	.794	67.789	1	.000	1.898	1.630	2.211
[stock movt r=0]	-.037	.0931	-.219	.146	.155	1	.694	.964	.803	1.157
[stock movt r=1]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.236[b]	.0293	.185	.301						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.667	.1553	.362	.971	18.438	1	.000	1.948	1.437	2.640
[feed=graz_cn]	.333	.2455	-.148	.814	1.844	1	.175	1.396	.863	2.258
[feed=graz_crp]	-.667	.4910	-1.629	.296	1.844	1	.175	.513	.196	1.344
[feed=graz_hy]	-.667	.3105	-1.275	-.058	4.609	1	.032	.513	.279	.944
[feed=graz_mn]	-.167	.2140	-.586	.253	.607	1	.436	.846	.556	1.288
[feed=grazing]	-.043	.1620	-.360	.275	.070	1	.791	.958	.697	1.316
[feed=gz_mn_hy0]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.217[b]	.0269	.170	.277						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.750	.2430	.274	1.226	9.529	1	.002	2.117	1.315	3.408
prod_sys=exten	-.139	.2468	-.623	.345	.317	1	.574	.870	.537	1.412
prod_sys=semi-inten	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.236[b]	.0293	.185	.301						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper	Hypothesis Test Wald Chi-Square	df	Sig.	Exp(B)	95% Wald Confidence Interval for Exp(B) Lower	95% Wald Confidence Interval for Exp(B) Upper
(Intercept)	.500	.1677	.171	.829	8.889	1	.003	1.649	1.187	2.290
[wat_rain=harvested]	.500	.5031	-.486	1.486	.988	1	.320	1.649	.615	4.420
[wat_rain=own_well]	.389	.2305	-.063	.841	2.847	1	.092	1.475	.939	2.318
[wat_rain=pan]	.064	.1795	-.288	.415	.126	1	.723	1.066	.750	1.515
[wat_rain=piped]	.500	.3211	-.129	1.129	2.424	1	.119	1.649	.879	3.094
[wat_rain=river]	.111	.1797	-.241	.463	.382	1	.536	1.118	.786	1.589
[wat_rain=well]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.225[b]	.0279	.176	.287						

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper	Hypothesis Test Wald Chi-Square	df	Sig.	Exp(B)	95% Wald Confidence Interval for Exp(B) Lower	95% Wald Confidence Interval for Exp(B) Upper
(Intercept)	.526	.0540	.421	.632	95.070	1	.000	1.693	1.523	1.882
[wat_dry= piped	.474	.2770	-.069	1.017	2.924	1	.087	1.606	.933	2.764
[wat_dry=own_well]	.248	.1003	.051	.444	6.109	1	.013	1.281	1.053	1.560
[wat_dry=pan	.105	.1207	-.131	.342	.761	1	.383	1.111	.877	1.408
[wat_dry=river	.474	.4737	-.455	1.402	1.000	1	.317	1.606	.635	4.064
[wat_dry=well	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.221[b]	.0275	.174	.282						

ii. Multivariate analysis

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	.473	.2033	.075	.872	5.419	1	.020	1.605	1.078	2.391
[ward=Ildamat]	-.049	.1273	-.299	.201	.148	1	.700	.952	.742	1.222
[ward=Iloodoki]	-.252	.1384	-.523	.020	3.304	1	.069	.778	.593	1.020
[ward=Kapu_N]	.158	.1425	-.121	.437	1.231	1	.267	1.171	.886	1.549
[ward=Ke_Poka]	.215	.1368	-.053	.483	2.476	1	.116	1.240	.949	1.622
[ward=Mata_So]	0[a]	.	.	.	.	.	.	1	.	.
[feed=graz_con]	.379	.2622	-.134	.893	2.094	1	.148	1.461	.874	2.443
[feed=graz_crp]	-.222	.4708	-1.144	.701	.222	1	.638	.801	.318	2.016
[feed=graz_hay]	-.398	.2993	-.984	.189	1.767	1	.184	.672	.374	1.208
[feed=graz_min]	-.028	.2125	-.444	.389	.017	1	.895	.972	.641	1.475
[feed=grazing]	.112	.1728	-.227	.450	.417	1	.519	1.118	.797	1.569
[feed=gz_mn_h]	0[a]	.	.	.	.	.	.	1	.	.
[wat_dry=0]	.389	.2719	-.144	.922	2.043	1	.153	1.475	.866	2.513
[wat_dry=own_well]	.119	.1069	-.091	.328	1.230	1	.267	1.126	.913	1.388
[wat_dry=pan]	-.013	.1278	-.264	.238	.010	1	.919	.987	.768	1.268
[wat_dry=river]	.464	.4397	-.398	1.326	1.114	1	.291	1.591	.672	3.766
[wat_dry=well]	0[a]	.	.	.	.	.	.	1	.	.
(Scale)	.185[b]	.0230	.145	.236						

Appendix 3: Ethical approval letter

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2020/255

Dr. Eunice Gathoni Mungai
University of Nairobi
Dept. PHP & T
08/01/2020

Dear Dr. Mungai

RE: Approval of proposal by Faculty of Veterinary Medicine, Biosafety, Animal use and Ethics committee

Investigation of *Toxoplasma gondii* and associated risk factors for infection of sheep and goats in Kajiado county, Kenya.

Dr. Eunice Mungai J56/ 11712/2018.

We refer to your MS.c proposal submitted to our committee for review and your application letter dated 13th December 2019. We have reviewed your application for ethical clearance for the study entitled **Investigation of *Toxoplasma gondii* and associated risk factors for infection of sheep and goats in Kajiado county, Kenya.**

The number of sheep and goats to be sampled is adequate. Blood collection and analysis meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines. The study is relevant in the Kenya context in identifying risk factors and creating awareness amongst the pastoral communities in Kenya.

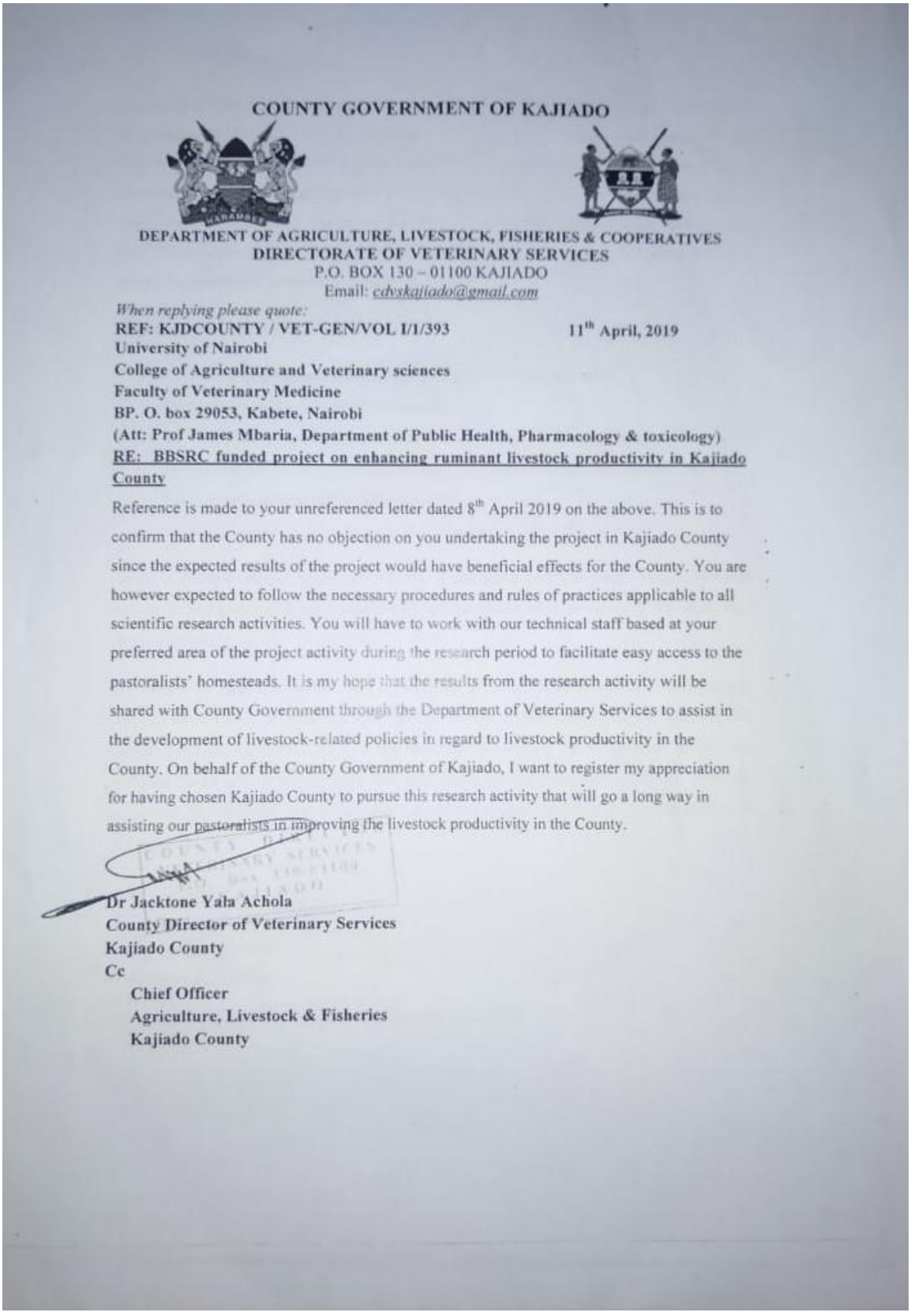
We have also noted that registered veterinary surgeons will supervise the work.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi.

Appendix 4: Approval letter from Director of veterinary services Kajiado



COUNTY GOVERNMENT OF KAJIADO

DEPARTMENT OF AGRICULTURE, LIVESTOCK, FISHERIES & COOPERATIVES
DIRECTORATE OF VETERINARY SERVICES
P.O. BOX 130 – 01100 KAJIADO
Email: *cdvskajiado@gmail.com*

When replying please quote:
REF: KJDCOUNTY / VET-GEN/VOL I/1/393 **11th April, 2019**

University of Nairobi
College of Agriculture and Veterinary sciences
Faculty of Veterinary Medicine
BP. O. box 29053, Kabete, Nairobi
(Att: Prof James Mbaria, Department of Public Health, Pharmacology & toxicology)

RE: BBSRC funded project on enhancing ruminant livestock productivity in Kajiado County

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

Dr Jacktone Yala Achola
County Director of Veterinary Services
Kajiado County

Cc
Chief Officer
Agriculture, Livestock & Fisheries
Kajiado County

Appendix 5: Research participants' consent form

Consent form

Project titled ***"Assessment and economic evaluation of sheep and goats reproduction efficiency, challenges and mitigation strategies in Kajiado, Kenya".***

Principal Investigators: Dr. Joshua Onono and Dr Pablo Alarcon
Organization: University of Nairobi (Kenya) and Royal Veterinary College (United Kingdom)
Project funded by the National Research Foundation (Kenya) and the Biotechnology and Biological Sciences Research Council (United Kingdom)

Part I: Information Sheet

Introduction

I am (name of the enumerator), working for the University of Nairobi. I am doing a research in shoats (sheep and goats) in Kajiado.
I am going to give you information and invite you to be part of this research. Before you decide, you can talk to anyone you feel comfortable with about the research.
This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them to me.

Purpose of the research

Effective reproduction of sheep and goats allow you to produce animals for food production, but also to ensure that you have new young animals each year to replace your old reproductive livestock (mothers). However, different challenges can interfere with the capacity of sheep and goats to get pregnant or to deliver a healthy newborn animal. These may have important consequences to you, such as not having enough offspring to sell or maintain your flock. For this reason, it is essential to identify those factors that may have a negative or positive impact on reproduction of your animals and to estimate how much of an impact these may have.

Type of Research Intervention

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

Participant Selection

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

and knowledge of sheep and goat reproduction and management.

Confidentiality

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

Sharing the Results

Nothing that you tell us today will be attributed to you by name. However, you can decide whether the results of the laboratory test will be communicated to the to the veterinary officers so they can implement the necessary measures to protect your family and your flock.

Voluntary Participation and Right to Refuse or Withdraw

Your participation in this research is voluntary, it is your choice whether to participate or not. You may change your mind later and stop participating even if you agreed earlier. You may stop participating in the interview at any time that you wish. I will give you an opportunity at the end of the interview to review your remarks, and you can ask to modify or remove portions of those, if you do not agree with my notes or if I did not understand you correctly.

The questions are not going to be about any sensitive or personal issues. However, you do not have to answer any question if talking about it makes you feel uncomfortable.

Benefits

You will not be provided any incentive to take part in the research and there will be not direct benefit to you, but your participation is likely to help us find out how to improve reproduction of your sheep and goats.

Who to Contact

If you have any questions, you can ask them now or later. If you wish to ask questions later, you may contact any of the following: *[Name and telephone number of contact from the University of Nairobi]*.

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

Questions to elucidate understanding:

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

Do you know what the study is about?

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

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

Part II: Certificate of Consent

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

An interview about reproductive performance in the small ruminant flock and challenges	☐
A blood sampling procedure in 6 ewes and 6 does of the flock	☐
I also give you permission to take some pictures of the animals and the farm	☐

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

Print Name of Participant_____
Signature of Participant _____
Date _____
Day/month/year

If illiterate [1]I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____
Signature of witness _____
Date _____
Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands what is going to be done.
I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.
A copy of this ICF has been provided to the participant.
Print Name of Researcher/person taking the consent_____
Signature of Researcher /person taking the consent_____
Date _____
Day/month/year

[1] A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). This informed consent was adapted from the Informed Consent Form Template for Qualitative Studies of the World Health Organization

Appendix 6: Data collection: Taking blood sample from a goat

Appendix 7: Gel electrophoresis of conventional PCR products

Appendix 8: Gel viewing after electrophoresis