SERO-PREVALENCE AND FACTORS ASSOCIATED WITH BRUCELLOSIS IN GOATS AND SHEEP AND ASSESSMENT OF PASTORALISTS, KNOWLEDGE ATTITUDE AND PRACTICES TOWARDS BRUCELLOSIS IN GARISSA COUNTY

MARK ODHIAMBO OBONYO

MASTER OF SCIENCE

(Applied Epidemiology)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY.

2018

Sero-prevalence and Factors Associated with Brucellosis in Goats and Sheep and Assessment of Pastoralists, Knowledge Attitude and Practices towards Brucellosis in Garissa County

Mark Odhiambo Obonyo

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

2018

DECLARATION

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

Signature: ..... Date: .....

Mark Odhiambo Obonyo

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

Signature: ..... Date: .....

Prof. Gideon Kikuvi, PhD

JKUAT, Kenya

Signature: ..... Date: .....

Dr. Kariuki Njenga, PhD

KEMRI, Kenya

DEDICATION

This work is dedicated to my wife Norah Mbithe, sons Israel and Ethan, my mother Dorcas, sisters Beatrice and Mercy and my brother Kennedy for their support, prayers, encouragement, and motivation to see this thesis become a reality.

ACKNOWLEDGEMENTS

First I would like to thank the almighty God for giving me strength and energy to accomplish this work. Secondly, I would wish to express my gratitude to my supervisors Prof. Gideon Kikuvi and Dr. Kariuki Njenga for their guidance and assistance during the research work.

I would like to also extend my sincere gratitude to Kenya Field Epidemiology and Laboratory Program (K-FELTP) for financial support to carry out the study. I would also wish to extend my sincere gratitude to the K-FELTP faculty members for their untiring support to ensure that I accomplish this work.

I also wish to acknowledge all the staff of the department of veterinary services and livestock production of Garissa County and staff at the Regional veterinary investigations Laboratory (RVIL) in Garissa and Central Veterinary Laboratory (CVL) in Kabete Nairobi for their testing and supply of reagents for sample testing.

I would also like to cooperation and efforts towards the success of this study especially in connection to sample acknowledge the study participants and the community leaders for support provided during the study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF EQUATIONS

LIST OF ACRONYMS AND ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
CFSPH	Center for Food security and Public Health
CFT	Complement Fixation Test
CI	Confidence Interval
CO_2	Carbon dioxide
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAO	Food and Agriculture Organization
H_2S	Hydrogen sulphide
ICSP	International Committee on Systematics of Prokaryotes
OIE	World Organization for Animal Health
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
RVIL	Regional Veterinary Investigations Laboratory
SLPS	Smooth Lipopolysaccharide
SPP	Species

WHO	World Health Organization
ZDU	Zoonotic Disease Unit, Kenya

ABSTRACT

Brucellosis, a zoonosis of major public health importance, is endemic in livestock in Kenya. Unfortunately, reliable data on Brucellosis in Kenya is scarce and data on sero-prevalence and risk factors associated with small ruminant Brucellosis in Garissa County is unknown. This was a cross-sectional study carried out to determine the sero-prevalence of Brucellosis and identify herd-level factors associated sero-prevalence in small ruminants in Garissa County of North Eastern Kenya. The study also assessed the pastoralists' knowledge, attitude and practices towards Brucellosis. A total of 2,400 sera from 120 flocks were collected from sheep and goats which were randomly selected using a multi-stage sampling technique and data on potential herd-level factors were collected from the pastoralists' ≥15 years using a pre-tested structured questionnaire. The sera were analyzed using Rose Bengal Plate Test (RBPT) and sero-positive reactors confirmed by Complement Fixation Test (CFT) using serial interpretation. A sample was considered to be positive when both tests results were positive and a herd was considered positive when a single animal within the herd tested positive on both tests. Multivariable logistic regression was used to investigate for independent factors associated with flock Brucellosis sero-positivity in small ruminants. The overall sero-prevalence of Brucellosis at individual animal-level was 20.0% (95% CI: 18.2% to 22.0%); in goats 24.3% (95% CI: 21.8% to 27.1%) and sheep 12.5% (95% CI: 10.2% to 15.2). Overall true herd-level sero-prevalence was 65.8% (95% CI: 54.3% to 77.2%). Seeking veterinary services [aOR=0.30 (95% CI: 0.12 to 0.76], introduction of new animals into the flock [aOR=8.0 (95% CI: 3.09 to 20.70] and experiencing abortions in the flock [aOR=3.43 (95% CI: 1.33 to 8.88] were independently associated with Brucellosis herd sero-prevalence in small ruminants. A total of 120 pastoralists were interviewed of which 95 (79%) had heard of Brucellosis and 17(18%) mentioned bacteria/germ as cause. Forty-four (46%) would do nothing if they had aborting animal in their herd, 91 (96%) consumed raw milk in the past year and 72 (76%) assisted an animal during parturition process and none used glove. The study highlights considerable high sero-prevalence of Brucellosis and factors that contributes for its

transmission in small ruminants in Garissa County. This poses potential public health threat associated with zoonotic transmission. The study also highlights that though the community has some knowledge on Brucellosis, attitudes and practices are poor. Enhanced public health education by the County government is recommended for effective prevention and control of Brucellosis in animals and humans in the area. Need to conduct animal-human linked study in the area for holistic understanding of epidemiology of the disease in the area.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Brucellosis is one of the world's most widespread zoonotic diseases caused by species of the genus *Brucella* (Moreno *et al.,* 2002). Brucellosis in sheep and goats occurs worldwide. The Mediterranean countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are especially affected. While *B. melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants (Omer *et al.*, 2000; Radostits *et al.*, 2007; Taleski *et al.*, 2002).

Brucellosis is a herd or flock problem. It is spread within the herd primarily by ingestion of contaminated material. Venereal infections can also occur, but this is mainly seen with *B. suis* infections. Congenital (*in utero*) or perinatal infections may also occur, with the ensuing development of latent infections. Spread between herds usually occurs by the introduction of asymptomatic chronically-infected animals. Brucellosis is a disease of the sexually mature animals with predilection for placentas, fetal fluids and testes of male animals. The disease in animals is transmitted by direct or indirect contact with infected or contaminated materials. Both wild and domestic animals are susceptible to infection with *Brucella* and may serve as carriers for other domestic animals. Initial infection in the reservoir species is often followed by abortion and subsequent delayed or permanent infertility. Infected animals shed the organisms in uterine discharges following abortion and subsequent parturition, and also in the colostrum and milk (Radostits *et al.,* 2007). Risk factors that have been identified to enhance the transmission of Brucellosis between and within herds include history of abortions within a herd, mixing of different animals during grazing and in watering places, introduction of new non-quarantined animals whose Brucellosis status is not known into the herd as

replacement stock or for breeding purposes and mixing with wild animals in watering and grazing places (Coelho *et al.,* 2008; Bamaiyi *et al.,* 2014; Sharifi *et al.,* 2014; Muma *et al.,* 2007a; Al-Majali *et al.,* 2007).

Transmission of infection to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted fetuses or placentas. Food-borne infection occurs following ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals. Occupational airborne infection in laboratories and abattoirs has also been documented. Accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis* Rev.1) can also occur, resulting in human infections. There are also case reports of venereal and congenital infection in humans (Al-Majali, 2005; Queipo-Ortuno *et al.,* 1997).

Human infections are characterized by a variable incubation period (from several days up to several months), and clinical signs and symptoms of continued, intermittent or irregular fever of variable duration, with headaches, weakness, profuse sweating, chills, depression and weight loss. Localized suppurative infections may also occur. The course of the disease can be variable especially in persons either not or inadequately treated (Al-Majali, 2005).

Diagnosis of clinical Brucellosis in humans and animals is initially made by use of appropriate serological or other immunological tests, and confirmed by bacteriological isolation and identification of the agent (OIE, 2009a).

Methods of prevention include health education to reduce occupational and food-borne risks, including pasteurization of all dairy products. However, education campaigns have never resulted in fully eliminating the risks of infection, and the ultimate prevention of human infection remains elimination of the infection among animals. This can be achieved by a combination of test and isolation/slaughter of positive animals, vaccination of susceptible animals and control of animal movements (Corbel, 1997).

Various countries all over the world have implemented different control policies using combinations of these control measures, with reported success in some countries and failure in others (Cutler *et al.,* 2005; Blasco, 1997).

1.2 Statement of the Problem

Brucellosis infection in sheep and goats causes heavy economic losses in animal production resulting from clinical disease, abortion, neonatal losses, reduced fertility, decreased milk production, slaughtering of the infected animals, cost of veterinary care, and replacement animals. In addition, the disease is an impediment to free animal movement and it is also a limiting factor for international trade of animals and their products (Radostits *et al*., 2007). The disease in humans is of great public health implication due to losses from high medical expenses, productivity losses and high costs of management of sequelae (Al-Majali, 2005).

In humans, Brucellosis, especially caused by *Brucella* melitensis, remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually. The true incidence of human Brucellosis worldwide is unknown and the estimated burden of the disease varies widely, from <0.03 to >160 per 100,000 population (Pappas *et al.,* 2006; Taleski *et al.,* 2002). The bacterial pathogen is classified by the US Centers for Disease Control and Prevention as a category (B) pathogen that has potential for development as a biological weapon with a potential of aerosol transmission (Seleem *et al.,* 2009).

In Kenya, there is no systematic control program for Brucellosis in livestock. Control program is based on individual farmer initiative and is based on testing ruminants more than 6 months of age with slaughter of serologically positives, and voluntary vaccination of calves using Brucella abortus S19 vaccine and Brucella melitensis Rev 1 vaccine for lambs and kids (McDermott and Arimi, 2002).

Brucellosis remains endemic in livestock and humans in several parts of Kenya and a hospital based study done in Garissa County among febrile patients, found a Brucellosis sero-prevalence of 31.8% and the true prevalence of 15.4% by PCR (Kiambi, 2014). Given that infected animals are the source of human infection, the increasing incidence of human Brucellosis probably reflects a similar trend in domestic animals. Animal Brucellosis has been reported every year particularly from the arid and semi-arid pastoral areas of the country (Kenya DVS Annual reports, 1999-2010). A study of Brucellosis in goats herds done in Kenya in 1976 in Garissa found a herd prevalence of 6.8% (McDermott and Arimi, 2002). In other parts of the world, a cross-sectional study to determine sero-prevalence of Brucella melitensis and to identify risk factors associated with goat sero-positivity in Michoacán, Mexico, blood samples were collected from 5,114 animals from 79 herds. Sera were tested for antibodies against *B. melitensis* using the Rose Bengal plate test (RBPT) and the complement-fixation test (CFT). A total of 55 herds out of the 79 herds tested had at least one seropositive animal. The animal-level true sero-prevalence was 9.8% (Solorio-Rivera *et al.,* 2007). In a study done in Spain using RBPT and CFT where 56 herds (35 ovine and 21 caprine) were sampled. Sixteen (29%) flocks (3 caprine and 13 ovine) were Brucellosis sero-positive. Overall, 0.7% of sheep and 0.1% of goats were sero-positive (Reviriego *et al*., 2000). In Africa, a study done in goats in Eastern and Western Uganda using Brucellosis card test (CT) and Tube Agglutination Test (TAT) in parallel, found an individual animal-level sero-prevalence of 4% and a herd-level sero-prevalence of 13% with CT; and a 13% animal-level sero-prevalence and 43% herd-level sero-prevalence with TAT (Kabagambe *et al.,* 2001). In Eriteria, a study using Rose Bengal Plate Test (RBPT) detected an animal-level sero-prevalence of 14.3% and a herd-level sero-prevalence of 56. 3% (Omer *et al.,* 2000).

In order for a Brucellosis control programme to be efficient, it is important to understand community local knowledge, attitudes and practices relating to Brucellosis to improve information delivery and initiate relevant control measures through disease education

among community participants (Smits, 2012). However in Kenya, there is scarcity of information on local community KAP on Brucellosis. A KAP study of Brucellosis conducted in Kenya among people with high level of contact with livestock found that the disease awareness and knowledge of the transmission routes were poor (Kangethe *et al.*, 2007).

1.3 Justification of the Study

Livestock plays a crucial role in the livelihood of the majority of residents of Garissa county who are predominantly pastoralists keeping mainly sheep, goats, cattle and camels. The pastoral production system is characterized by pastoralists keeping relatively large herds that graze freely in vast communal lands with watering points. Livestock owners practice a free grazing system, using communal grazing grounds and watering points where cattle and small ruminants graze separately. The majority of herd owners are semi-sedentary with only a few still practicing nomadism. Animals are usually kept in the animal shade (boma) at night and young stock of usually less than two months of age share the house with humans. Older cattle are kept in a separate shade with sheep and goats. Livestock owners also keep relatively large herds and flocks for meat and milk for their families, as a source of savings, and to meet some cultural and social values such as dowry, celebrations and gifts and for social prestige. It is has been estimated that more than 95% of the household income is derived from livestock and livestock products (Garissa, 2013). However this dependence on livestock makes people vulnerable to zoonotic diseases. Some of the socio cultural practices such as consumption of raw milk make residents of Garissa at greater risk of infection with Brucellosis.

Although there are no published studies that incriminate *Brucella* spps as cause of abortions in goats and sheep in Garissa, Brucellosis has been suspected on basis of clinical grounds by local veterinary officers working in Garissa County. Unfortunately,

reliable data on Brucellosis in Kenya is scarce and data on sero-prevalence and risk factors associated with sheep and goat Brucellosis in Garissa County is not known.

Therefore a better understanding of the sero-prevalence of Brucellosis and associated herd-level risk factors for exposure across different geographical areas in Kenya is important in generating evidence based information that may help towards formulating control strategies especially in livestock and this will impact in reducing the incidence of infection in both livestock and humans. Control of Brucellosis in Garissa County is important in safeguarding the livelihoods of majority of people in Garissa County who largely depends on livestock keeping, enhancing food security in the region and in long term promoting good health and wellbeing of people living in Garissa which directly linked to the sustainable development goal 3 (SDG3).

Improvement of knowledge, attitudes and practices among pastoralists could have a significant impact on the reduction of many zoonotic infections including Brucellosis (Kangethe *et al.*, 2007).

1.4 Research questions

1. What is the individual animal-level and herd-level sero-prevalence of Brucellosis in goats and sheep in Garissa County?
2. What are the herd-level factors associated with Brucellosis sero-prevalence in Garissa County?
3. What are the farmer's knowledge, attitude and practices regarding Brucellosis in Garissa County?

1.5 Objectives

1.5.1 Broad objective

To evaluate individual animal-level and herd-level sero-prevalence, determine herd-level factors associated with Brucellosis in goats and sheep and assess farmer's knowledge, attitude and practices regarding Brucellosis in Garissa County.

1.5.2 Specific objectives

1. To determine individual animal-level and herd-level sero-prevalence of Brucellosis in goats and sheep in Garissa County.
2. To determine herd-level factors associated with Brucellosis sero-prevalence in goats and sheep in Garissa County.
3. To evaluate pastoralists knowledge, attitude and practices regarding Brucellosis in Garissa County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Etiology of Brucellosis

Ten *Brucella* spps are currently recognized, seven of them that affect terrestrial animals are: B. *abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae,* and *B. microti* (Scholz *et al.,* 2008) and two that affect marine mammals are: *B. ceti* and *B. pinnipedialis* (Foster *et al.,* 2007) and B. inopinata isolated from a breast implant wound of a woman with clinical signs of Brucellosis (Scholz *et al.,* 2010). Recently, a novel *Brucella* spps has been isolated from postpartum uteruses of pregnant baboons with history of stillbirth (Schlabritz-Loutsevitch, 2009).The newly isolated *Brucella* spps has not yet been classified nor included in the above mentioned list. The first three species are called classical *Brucella* and within these species, seven biovars are recognized for *B. abortus*, three for *B. melitensis* and five for *B. suis*. The remaining species have not been differentiated into biovars.

Brucellosis in sheep and goats is mainly caused by *Brucella melitensis,* containing three biovars (biovars 1, 2 and 3). All three biovars cause disease in small ruminants, but their geographic distribution varies. *Brucella abortus* and *Brucella suis* infections also occur occasionally in small ruminants, but clinical disease seems to be rare (CSFPH, 2009). *B. ovis* infections occur in sheep in sub-Saharan Africa and have been associated with epididymitis, orchitis and infertility in rams (McDermott and Arimi, 2002).

2.2 Morphology of *Brucella* spps

Brucella spps are gram-negative cocco-bacilli or short rods 0.6 to 1.5 μm long by 0.5 to 0.7 μm in width, arranged singly and less frequently in pairs or small groups. They are

non-spore-forming and non-capsulated. Although they are described as non-motile, they carry all the genes except the chemotactic system, necessary to assemble a functional flagellum (Fretin *et al.,* 2005). *Brucella* usually does not show bipolar staining. They are not truly acid-fast but resist discoloration by weak acids, thus stain red by the Stamp's modification of Ziehl-Nielsen method, which is sometimes used for the microscopic diagnosis of Brucellosis from smears of solid or liquid specimens. The morphology of *Brucella* spps is fairly constant except in old cultures, where pleomorphic forms may be evident (Godfroid *et al.,* 2005; OIE, 2009a).

On suitable solid media *Brucella* colonies are visible after 2 days incubation. After 4 days incubation, *Brucella* colonies are round, 1-2 mm in diameter, with smooth (S) margins, translucent and a pale honey color when plates are viewed in the daylight through a transparent medium. When viewed from above, colonies appear convex and pearly white. Later, colonies become larger and slightly darker. Smooth *Brucella* cultures, especially *B. mellitensis* cultures, have a tendency to undergo variation during growth, especially with subcultures, and dissociate to rough (R) forms, and sometimes mucoïd (M) forms. Colonies are then much less transparent with a more granular, dull surface (R) or a sticky glutinous texture (M), and range in color from matt white to brown in reflected or transmitted light. Intermediate (I) forms between S, R and M forms may occur in cultures undergoing dissociation to the non-smooth state. Changes in the colonial morphology are generally associated with changes in virulence, serological properties and phage sensitivity. (Alton *et al.,* 1988; Quinn *et al.,* 1999; OIE, 2009a; Godfroid *et al.,* 2010).

2.3 Taxonomy of Brucella spps

Due to high degree of DNA homology in DNA-DNA hybridization assays (>90% identity), including the recently recognized marine mammal strains , it was proposed that the genus *Brucella* should be a monospecific genus, with *B. melitensis* as the sole species and the other species should be considered as biovars (Verger *et al.,* 1985, 1987,

1998, 2000; Xavier, 2009). Conversely, several molecular genotyping methods have been developed and applied to characterize *Brucella* spps, indicating that significant DNA polymorphisms occur between species, which favor the current multi-species classification of *Brucella* (Halling, 2005). Importantly, comparison of genome sequences of *B. suis* and *B. melitensis* demonstrated that exist clusters of genes that are unique in both species (designated genetic islands). It is reasonable to hypothesize that these unique genes may contribute to the differences in host specificity between *Brucella* spps (Tsolis, 2002). Furthermore, recent studies based on comparative whole genome analysis of several *Brucella* spps indicate that there is limited divergence with a large number of pseudo genes. Interestingly, these genomic analyses do not clearly explain the host preferences of *Brucella* spps (Wattam, 2009; Foster, 2009). One of these studies indicates that at the *B. ovis* is the basal lineage to the rest of the *Brucella* spps and that apparently most *Brucella* spps diverged from their common *B. ovis* ancestor in the past 86,000 to 296,000 years (Foster, 2009).

The International Committee on Systematics of Prokaryotes (ICSP), Subcommittee on the taxonomy of *Brucella* recommended a taxonomic classification that includes different species within the genus, either classical or new, which are still considered as individual species. Therefore, the genus currently group ten species, namely *B. melitensis, B. abortus, B. suis, B. neotomae, B. ovis, B. canis, B. ceti, B. pinnipedialis, B. microti and B. innopinata* (ICSP, 2010).

B. ovis and *B. canis* have only been encountered in the rough form whereas the rest of the species occurs in smooth form. Three biovars are recognized for *B. melitensis* (1-3), seven for *Brucella abortus* (1-6 and 9), and five for *Brucella suis* (1-5) (Godfroid *et al.*, 2010).

2.4 History and importance of *Brucella melitensis*

Sheep and goats Brucellosis (excluding *Brucella ovis* infection which is rarely pathogenic for humans) is a zoonotic infection with important effects on both public health and animal health and production and is widespread in many areas of the world, particularly in some Mediterranean and Middle Eastern countries. *Brucella mellitensis*, the main etiologic agent of Brucellosis in small ruminants, was the first species in the genus *Brucella* described. It was first isolated by Bruce in 1887 from the spleens of soldiers dying of Mediterranean fever on the island of Malta. Bruce called it *Micrococcus melitensis*. The origin of the disease remained a mystery for nearly 20 years until it was discovered that goats were the source of infection for human population (Nicoletti, 2002).

2.5 Brucella melitensis infection in sheep and goats

The major route of infection is through the mucous membranes of the oropharynx and upper respiratory tract or the conjunctiva following contact with contaminated pastures. Other potential routes of infection are through the mucous membranes of the male or female genital tract. After gaining entrance to the body, the organisms encounter the cellular defenses of the host, but generally succeed in arriving via the lymph channels at the nearest lymph node. The fate of invading bacteria is mainly determined by the cellular defenses of the host, chiefly macrophages and T lymphocytes, though specific antibody undoubtedly plays a part. The outcome depends on the ruminant species infected, age, immune status of the host, pregnancy status, and the virulence and number of the invading *Brucella*. When the bacteria prevail over the body defenses, a bacteremia is generally established. This bacteremia is detectable after 10 to 20 days and persists from 30 days to more than two months. If the animal is pregnant, bacteremia often leads to the invasion of the uterus. At the same time, infection becomes established in various lymph nodes and organs, often in the udder and sometimes in the spleen. During this first stage of infection, the major clinical sign is abortion but other signs due to a

localization of *Brucella* may be observed i.e. orchitis, epididymitis, hygroma, arthritis, metritis, subclinical mastitis, occasionally retained placenta. However, numerous animals develop self-limiting infections or they become asymptomatic latent carriers. Abortion generally does not occur if the female becomes infected at the third trimester of pregnancy (Bishop *et al., 1994*; Radostits *et al., 2007*; CSFPH, 2009)

The second stage is characterized by either elimination of *Brucella* or, more frequently, by a persistent infection of mammary glands and supramammary and genital lymph nodes with constant or intermittent shedding of the organisms in the milk and genital secretions (Radostits *et al., 2007;* Benkirane. A, 2006)

Animals generally abort once during the second trimester, but re-invasion of the uterus occurs in subsequent pregnancies with shedding in fluids and membranes. The pregnancy can also continue to full-term. The proportion of newly infected females that abort varies with the circumstances. The percentage of infected females lambing/kidding in a flock may reach 40% (Radostits *et al.,* 2007). Females that are born into an infected environment and subsequently infected generally abort less frequently as compared to those born in uninfected environment but later get infected. This explains the high level of abortions in newly infected flocks and their relatively low frequency in flocks where infection is enzootic (Radostits *et al.,* 2007). Greatly reduced milk yield follows abortion, and infection of the udder following a normal birth also leads to a considerable reduction in yield. In spite of this, clinical signs of mastitis are seldom detectable in naturally infected goats (Alton 1990; Radostits *et al.,* 2007; Godfroid *et al.,* 2005).

2.6 Pathological lesions in animals

Brucella-infected animals generally develop granulomatous inflammatory lesions which frequently are found in lymphoid tissues and organs such as reproductive organs, udder and supramammary lymph nodes and sometimes joints and synovial membranes. The lesions when present are not pathognomonic. The following could be observed:

necrotizing placentitis, palpable testicular alterations, necrotizing orchitis and epididymitis with subsequent granulomatous, necrotizing seminal vesiculitis and prostatitis. Acute mastitis with palpable nodules and the production of clotted and watery milk may occur. Some aborted fetuses may have an excess of blood-stained fluids in the body cavities, with enlarged spleen and liver. Others appear normal. Infected fetal membranes show changes affecting part or all of the membrane. The necrotic cotyledons lose their blood-red appearance becoming edematous and dull-grey in color (Radostits *et al.,* 2007; Godfroid *et al.,* 2004).

2.7 Brucella melitensis infection in humans

Human Brucellosis is widely distributed all over the world, with regions of high endemicity such as Mediterranean, Middle East, Latin America, parts of Asia and Africa (Corbel, 1997). The incubation period of Brucellosis normally is 1–3 weeks, but it can be several months before showing signs of infection. *B. melitensis* is associated with acute infection whereas the infections with other species are usually sub-acute and prolonged (Mantur *et al.,* 2007). *B. melitensis* is the most virulent *Brucella* for humans with a few organisms (10 to 100) being sufficient to cause a debilitating infection (Fugier *et al.,* 2007).

The World Health Organization (WHO) laboratory biosafety manual classifies *Brucella* (and particularly *B. melitensis*) in risk group III (OIE, 2009b). Humans acquire Brucellosis mainly through ingestion of contaminated milk and unpasteurized dairy products. Contact of mucosa and skin abrasions with fluids and tissues from aborted fetuses of infected animals are also important sources of *Brucella* transmission (Fugier *et al.,* 2007). Furthermore, people may be infected by inhalation of contaminated dust or aerosols. Thus, *Brucella* is one of the most common laboratory acquired pathogens worldwide and is included in the potential biological weapon list (Xavier, 2009).

Humans are accidentally infected and almost always are dead-end hosts of *Brucella* infections. The disease is primarily an occupational risk in exposed professions, *i.e*. veterinarians, farmers, laboratory technicians, abattoir workers, and others who work with animals and their products. People living near infected premises may also contract infection. The primary source is the animal and infection is contracted either by direct or indirect contact through the skin or mucous membranes or ingestion of contaminated products, especially fresh dairy products. The maximum danger is therefore during the lambing or kidding period. Dairy products are the main source of infection for people who do not have direct contact with animals. However much of the milk which is consumed is nowadays rendered safe by pasteurization or boiling, but cheese made from sheep and goat milk is preferably prepared from untreated milk may come from *Brucella* infected animals. During the course of cheese manufacture, any *Brucella* present in the milk become trapped in the clot and thus concentrated in the cheese, although bacteria may subsequently be inactivated by manufacturing or ripening processes. The prevalence of human Brucellosis acquired from dairy products is seasonal, reaching a peak soon after kidding and lambing. Abattoir workers handling infected animals are also at risk, especially from the contents of uteri and udders. The handling of raw wool has been identified as a potential source of infection of workers involved. *B. melitensis* is also easily acquired by laboratory infection (Kangethe *et al.,* 2007; OIE, 2009a; OIE, 2009b).

Human infections with *B. melitensis* have variable clinical manifestations and can become life threatening (Colmenero, 2002). Although the majority of patients present with general symptoms, such as fever, malaise, sweats and lymphadenopathy and/or hepatosplenomegaly, a more severe form of the disease can be accompanied with osteo-articular signs (spondylitis, arthritis and osteomyelitis) or *genitourinary* tract *changes* (orchitis, epididymitis, glomerulonephritis and kidney abscesses) (Colmenero, 2002).

Human Brucellosis is also known for complications and involvement of internal organs and its symptoms can be very diverse depending on the site of infection and include

encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis (Acha and Szyfres, 2003). Spontaneous abortions, mostly in the first and second trimesters of pregnancy, are seen in pregnant women infected with *Brucella* (Khan *et al.*, 2001). Although rare complication, Brucella endocarditis (<2% of cases) is most commonly associated with *B. melitensis* infection and is the most severe complication. It accounts for at least 80% of deaths due to Brucellosis (Reguera *et al.*, 2003). Lack of appropriate therapy during the acute phase may result in localization of *Brucella* in various tissues and organs and lead to sub-acute or chronic disease that is very hard to treat (Young, 1995). Nervous, genitourinary, hepatosplenic and cardiovascular complications been observed. Brucellosis is termed *chronic* when it includes one or more of the signs described above and persists or recurs over a period of six months or more. *Brucella* dermatitis traditionally known as "allergy" to *Brucella* has also been associated with *B. melitensis.* Symptoms and signs of Brucellosis usually referred to as fever of unknown origin can be confused with other diseases including enteric fever, malaria, rheumatic fever, tuberculosis, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumors (Mantur *et al.*, 2007). Live animal vaccines *B. melitensis* Rev. 1 and B. abortus strain 19 are known to cause disease in humans. The course of the disease with vaccine strains is usually shorter and more benign (Acha and Szyfres, 2003). Direct person-to-person spread of Brucellosis is extremely rare. Mothers who are breast-feeding may transmit the infection to their infants and sexual transmission has also been reported (Carrera *et al.*, 2006; Kato *et al.*, 2007).

2.8 Diagnosis of Brucellosis in sheep and goats

2.8.1 Clinical signs of Brucellosis in sheep and goats

Brucellosis should be considered in flocks and herds with history of abortions and stillbirths occurring without concurrent illness. Other diseases causing abortion in small ruminants, particularly chlamydiosis and coxiellosis, should be considered. *B. ovis* can also cause epididymitis and orchitis in rams (CSFPH, 2009).

2.8.2 Staining and microscopy of Brucella organisms

Microscopic examination of smears stained with the Stamp's modification of the Ziehl-Neelsen method can be useful for a presumptive diagnosis, particularly if the direct examination is supported by serology. *Brucella* spps are not truly acid-fast, but they are resistant to decolorization by weak acids, and stain red against a blue background. *Brucella* organisms are coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. This test is not definitive. Other organisms that cause abortions such as *Chlamydophila abortus* and *Coxiella burnetii* can resemble *Brucella. B. ovis,* which causes epididymitis and orchitis in rams, can also be confused with *B. melitensis.* Immuno-staining is sometimes used to identify *Brucella* in smears (Alton *et al*., 1988).

2.8.3 Serology of Brucellosis

Serology can be used for a presumptive diagnosis of Brucellosis, or to screen flocks. Serological tests are not completely specific and cannot always distinguish reactions due to *B. melitensis* from cross-reactions to other bacteria, particularly *Yersinia enterocolitica* O: 9. The most commonly used serological tests in small ruminants are the buffered *Brucella* antigen tests, the card agglutination test (CAT) and rose bengal plate agglutination tests(RBPT) and the complement fixation test (CFT). Indirect or competitive enzyme-linked immunosorbent assays (ELISAs) are also used. A brucellin allergic skin test is sometimes used to test unvaccinated sheep and goats for *B. melitensis*. This test is performed by injecting the allergen into the lower eyelid resulting into delayed type hypersensitivity characterized by a local swelling and induration (OIE, 2009a, b; CSFPH, 2007).

2.8.3.1 Antigenic cross reactions of *Brucella* organisms

All smooth *Brucella* strains show complete cross-reaction with each other in agglutination tests with unabsorbed polyclonal antisera, a cross-reaction which does not

extend to non-smooth variants. Cross-reactions between non smooth strains can be demonstrated by agglutination tests with unabsorbed anti-R sera. Lipopolysaccharide (LPS) comprises the major surface antigens of the corresponding colonial phase involved in agglutination. The S-LPS molecules carry the A and M antigens, which have different quantitative distribution among the smooth *Brucella* strains. This is of value in differentiating biovars of the major species using absorbed monospecific A and M antisera. Serological cross-reactions have been reported between smooth *Brucella* and various other Gram negative bacteria, e.g. *Escherichia coli* O: 116 and O: 157, *Salmonella* group N (O: 30) of Kaufmann-White, *Pseudomonas multophilia*, *Vibrio cholera* and especially *Yersinia enterocolitica* O: 9. These organisms can induce significant levels of antibodies which cross react with S-LPS *Brucella* antigens in diagnostic tests (Nielsen *et al.,* 2006; Munoz *et al.,* 2005; CSFPH, 2007).

2.8.4 Culture of Brucella organisms

A definitive diagnosis can be made if *B. melitensis* is cultured from an animal. *Brucella* spps can be isolated on a variety of plain media, or selective media. Vaginal swabs and milk samples are the best samples to isolate *B. melitensis* from live sheep and goats. *B. melitensis* can also be cultured from aborted fetuses (stomach contents, spleen and lung) or the placenta. The spleen, mammary and genital lymph nodes, udder and late pregnant or early post-parturient uterus are the most reliable samples to collect at necropsy. This organism can also be cultured from semen, the testis or epididymis, and arthritis or hygroma fluids.

Brucella spps are aerobic, but some strains require an atmosphere containing 5-10% carbon dioxide (CO_2) added for growth, especially on primary isolation e.g. *B. abortus* wild type (biovars 1-4). Others, like B. abortus wild type (biovars 5, 6, 9), B. abortus S19 vaccine strain, *B. melitensis*, and *B. suis*, do not require CO_2 for growth. The optimum pH for growth varies from 6.6 to 7.4, and culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36-38°C,

but most strains can grow between 20°C and 40°C. *Brucella* requires biotin, thiamin and nicotinamide. The growth is improved by serum or blood, but haemin (V-factor) and nicotinamide-adenine dinucleotide (X-factor) are not required. The growth of most *Brucella* strains is inhibited on media containing bile salts, tellurite or selenite. Growth is usually poor in liquid media unless culture is vigorously agitated. Growth in static liquid media favors dissociation of smooth-phase cultures to non-smooth forms. Continuous and vigorous aeration will prevent this, provided a neutral pH is maintained. In semi solid media, CO_2-independent *Brucella* strains produce a uniform turbidity from the surface down to a depth of a few millimeters, while cultures of CO_2-requiring strains produce a disk of growth a few millimeters below the surface of the medium (Godfroid *et al.,* 2010; OIE, 2009a, b, CSFPH, 2007).

Since *B. melitensis* does not require serum or CO2 for growth and can be isolated on ordinary solid media under aerobic conditions at 37°C, however, the use of nonselective media cannot be recommended because of the risk of overgrowing contaminants usually present in field samples. Selective media are needed for isolation purposes. The Farrell's selective medium, developed for the isolation of *B. abortus* from milk (Farrell, 1974), is also recommended for the isolation of *B. melitensis* (Alton *et al.,* 1988). However, nalidixic acid and bacitracin, at the concentration used in this medium, may have inhibitory effects on some *B. melitensis* strains (Marín *et al.,* 1996b). Thus, its sensitivity for the isolation of *B. melitensis* from naturally infected sheep is sometimes lower than that obtained with the less selective Thayer-Martin's modified medium (Marín *et al.,* 1996a). The sensitivity of bacteriological diagnosis is significantly increased by the simultaneous use of both the Farrell's and the modified Thayer-Martin's media (Marín *et al.,* 1996b). Non selective, biphasic medium, known as Castaneda's medium, is recommended for the isolation of *Brucella* from blood and other body fluids or milk, where enrichment culture is usually advised. Castaneda's medium is used because *Brucella* tends to dissociate in broth medium, and this interferes with bio-typing by conventional bacteriological techniques. *B. melitensis* can be identified to the species

and biovars level by phage typing and cultural, biochemical and serological characteristics. Genetic techniques can also be used for bio-typing. Biotyping of *Brucella* spps is performed using different tests, the most important being agglutination tests with antibodies against rough or smooth LPS, i.e. against the A or M epitopes of 'O' chain polysaccharides (O-LPS); lysis by phages, dependence on CO2 for growth, measured usually in primary cultures; production of H2S; growth in the presence of basal fuchsine or thionine; and the crystal violet or acriflavine tests (Alton *et al.*, 1988). The vaccine strain (*B. melitensis* strain Rev.1) can be distinguished from field strains by its growth characteristics and sensitivity to antibiotics and other additives (Godfroid *et al.*, 2010; OIE, 2009a, b).

2.8.5 Biochemical tests for Brucella organisms

The metabolism of *Brucella* is oxidative and *Brucella* cultures show no ability to acidify carbohydrate media in conventional tests. The *Brucella* spps are catalase positive and usually oxidase positive, and they reduce nitrate to nitrite (except *B. ovis* and some *B. canis* strains). The production of H_2S from sulphur containing amino-acids also varies. *B. melitensis* does not produce H_2S. Urease activity varies from fast to very slow. Indole is not produced from tryptophane and acetyl-methyl-carbinol is not produced from glucose (Alton *et al.,* 1988; Quinn *et al.,* 1999; Corbel *et al.,* 2005; Godfroid *et al.,* 2010).

2.8.6 Susceptibility to phages by Brucella organisms

Over 40 *Brucella* phages have been reported to be lytic for *Brucella* members. All phages are specific for the genus *Brucella*, and are not known to be active against any other bacteria that have been tested. Thus, lysis by *Brucella* phages is a useful test to confirm the identity of *Brucella* spps and for speciation within the genus. The *Brucella* phages currently used for

Brucella typing are: Tbilisi (Tb), Weybridge (Wb), Izatnagar1 (Iz1) and R/C.

The three former phages are used for differentiation of smooth *Brucella* spps. R/C is lytic for *B. ovis* and *B. canis* (OIE, 2009a).

2.8.7 Other methods for detection of Brucella organisms

Animal inoculation is uncommonly used for isolation, but occasionally necessary when other techniques fail. Guinea pigs or mice can be used (CSFPH, 2009).

Several PCR based methods have also been developed. The best validated methods are based on the detection of specific sequences of *Brucella* spps such as the 16S-23S genes, the *IS*711 insertion sequence or the *bcsp*31 gene encoding a 31-kDa protein (Ouahrani-Bettache *et al.*, 1996; Baddour and Al-Khalifa, 2008).

2.8.8 Sero-prevalence of Brucellosis in small ruminants

In a study conducted in Jordan on ovine and caprine Brucellosis (Brucella melitensis) in aborted animals in Jordanian sheep and goat flocks, 255 biological samples were collected from188 animals (81 sheep and 107 goats) during the lambing season from September 2009 to April 2010 from the Mafraq region of Jordan. Sampled animals belonged to 93 sheep and goat flocks that had abortion cases in the region. One hundred and seven (41.9%) biological samples were positive for the omp2 primers that were able to identify all Brucella species in the collected samples which were obtained from 86 aborted animals (86/188 = 45.7%). These positive samples were obtained from 28 sheep and 33 goats. The prevalence rate of B. melitensis was 27.1% (51/188) among aborted animals (Samadi *et al.*, 2010).

A survey to estimate the seroprevalence of ovine and caprine brucellosis was conducted in the region of Trás-os-Montes e Alto Douro, Northeast of Portugal. In total, 278,097 small ruminants and 5,466 flocks from 13 Livestock Farmers Organizations (OPP's)

were analyzed. A total of 487 (8.9%) flocks had one or more serologically positive animals with values ranging between 8.2% and 9.7%. The individual seroprevalence was 0.44%. There were significant differences in seroprevalence rates among herd sizes, species and constitution of herd, production's type and OPP (Coelho *et al.,* 2013).

In Iran, a cross-sectional study conducted to estimate seroprevalence and to identify flock-level factors associated with seropositivity to Brucellosis in small ruminants in Kerman province, southeastern Iran. In October-November 2011, serum samples were randomly collected from 1767 sheep and 1233 goats, older than 18 months, from 300 flocks. The sera were initially screened for the presence of anti-Brucella antibodies using the Rose-Bengal test; those found to be positive were then examined by Wright and 2-mercaptoethanol Brucella agglutination tests. A total of 63 (21%) flocks had at least one seropositive animal (Sharifi *et al.,* 2014).

In a cross-sectional study done in Ethiopia in in South Omo Zone of Southern Ethiopia in goats, out of 384 tested animals; 20 (5.2%) of them were found positive for RBPT. Up on further testing the RBPT positive sera by CFT, 16 (4.2%) were found seropositive. Thus, overall number of seropositive goats in selected areas of South Omo Zone was 16 (4.2%) after RBPT and CFT. This study also found that Seroprevalence of caprine brucellosis was significantly associated with abortion rate in the animals (Ashagrie et al., 2011). In another cross-sectional study done in Somali Regional State, Eastern Ethiopia, sera samples were screened by Rose Bengal Plate Test (RBPT), and all samples tested positive by the RBPT were subjected to Complement Fixation Test (CFT) for confirmation and a total of 730 sera samples (421 of sheep and 309 of goats) were tested of which 12 serum samples that were positive by RBPT, 11 were positive by CFT (Bekele *et al.,* 2011). Yet in another cross sectional study carried out from October 2008 to April 2009 to determine the sero-prevalence of brucellosis in small ruminants in and around Bahir Dar, northwest Ethiopia, a total of 500 serum samples (270 from sheep and 230 from goats) were collected from extensive management system with no history of vaccination. All serum samples were initially screened by Rose-Bengal-Plate Test

(RBPT) and positive reactors to RBPT (n=6) were further tested by complement fixation test (CFT) for confirmation of which 2 (0.4%) turned positive (Ferede *et al.,* 2011). In another study conducted in Ethiopia in Dire Dawa region Eastern Ethiopia in small ruminants, out of 384 sheep and goats sera tested using Rose Bengal plate test (RBPT) and complement fixation test (CFT), 36 (9.38%) reacted positively using RBPT. Of these reactant sera, 35 also tested positive using CFT, giving an overall prevalence of small ruminant brucellosis of 9.11% (Negash *et al.,* 2012). In another sero-prevalence study on small ruminant Brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia, Sera from 2000 sheep and goats were tested by Rose Bengal Plate test (RBPT) and Indirect Enzyme Linked Immuno – Sorbent Assay (I-ELISA). Out of the 2000 sera tested 1.9 % (n= 38) were positive to RBPT and 9.7 % (n=193) were positive to I – ELISA. Brucella antibodies were more prevalent in goats (13.2%) as compared to sheep (5.6%). This difference was statistically significant ($X^2 = 32.5$; $P < 0.001$) (Teshale *et al.,* 2006).

In a study conducted in Niger to determine sero-prevalence and potential risk factors for *Brucella*

Spps infection in traditional cattle, sheep and goats reared in urban, peri-urban and rural areas, 5,192 animals from 681 herds were included in the study. Serum samples and hygroma fluids were collected and tested for Brucellosis antibodies using indirect ELISA. The true adjusted herd-level sero-prevalence of Brucellosis ranged between 11.2% and 17.2% and the true adjusted animal-level sero-prevalence was 1.3% (Boukary *et al*., 2013).

2.8.9 Risk factors for small ruminant Brucellosis

In a cross-sectional study conducted in Iran to determine risk factors of small ruminant brucellosis in Southeast Iran in 2012, after adjustment for the sampling fraction, a multivariable multi-level logistic model was used to detect the potential risk factors of

the infection. The final model identified presence of purchased animals (OR=8.39; 95% CI: 1.10-64.90) as an independent factor associated with Brucellosis sero-prevalence (Sharifi *et al.*, 2012; Sharifi *et al.*, 2012).

In a case control study conducted in Mexico to determine risk factors for brucellosis seropositivity of goat herds in the Mexicali valley of Baja California, Mexico, an important risk factor for goat herd brucellosis seropositivity in the Mexicali Valley was importation of goats from another state in Mexico. This was explained to be attributed to transportation of goats from higher-prevalence regions of the country to Mexicali which is a low-prevalence region (Mikolon *et al.*, 1998).

In a cross-sectional study conducted in Spain to determine risk factors for Brucellosis sero-prevalence of sheep and goat flocks in Spain, Eleven risk factors were studied at the group-level by logistic regression using flock brucellosis-status as outcome, and by linear regression using percentage of brucellosis-seropositivity as outcome. Both final models contained the same variables: contact with sheep and grazing in communal pastures as risk factors, and frequency of disinfecting practices as a protective factor (Reviriego *et al*., 2000).

In a study conducted in Niger to determine sero-prevalence and potential risk factors for *Brucella*

Spps infection in traditional cattle, sheep and goats reared in urban, peri-urban and rural areas, (Boukary *et al*., 2013). At herd level, the risk of transmission was increased by transhumance (OR of 5.4; 95% CI: 2.84–10.41), the occurrence of abortions (OR of 3.0; 95% CI: 1.40–6.41), and for herds having more than 50 animals (OR of 11.0; 95% CI: 3.75–32.46) (Boukary *et al*., 2013).

2.8.10 Knowledge attitude and practices towards Brucellosis

In Africa, a KAP study on Brucellosis conducted in Egypt showed a relative high general knowledge of Brucellosis but still a high-risk behavior among livestock owners which the authors concluded might contribute to a high sero-prevalence of brucellosis among livestock in the area (Holt *et al*., 2013). Another study done in Uganda found that respondents had moderate overall knowledge of human and animal Brucellosis and agro-pastoralists were found to have better knowledge compared to the pure pastoralists (Kansiime *et al*., 2014).

2.8.11 Conceptual framework

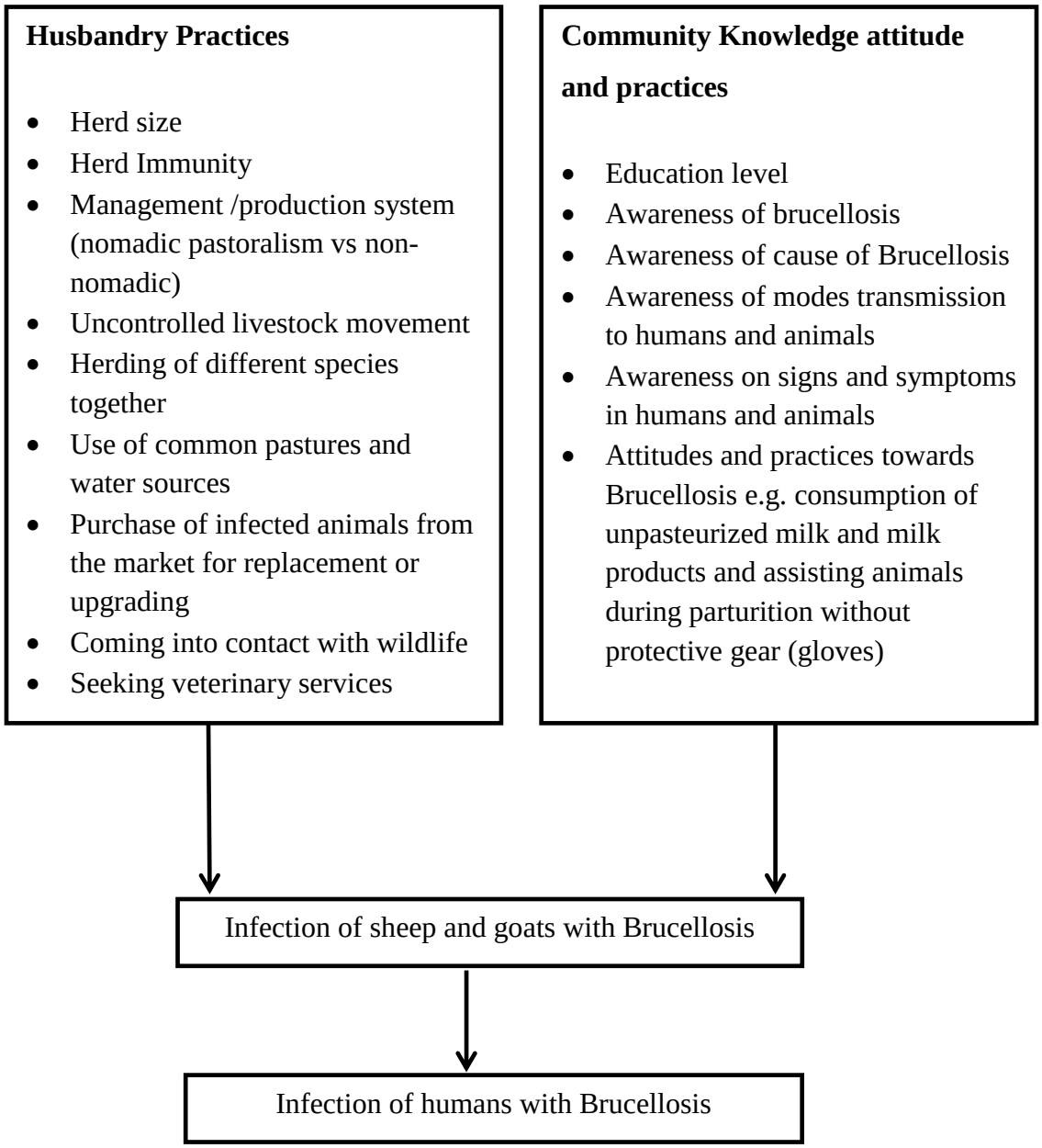

Figure 2.1: Conceptual Framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Garissa County is one of the three counties of the original North Eastern Province. The County is further divided into seven sub-counties namely: Garissa Township, Balambala, Lagdera, Dadaab, Fafi, Ijara and Hulugho. It boarders Isiolo county to the Northwest, Wajir county to the North and Republic of Somalia to the East, Tana river county to the west and Lamu county to the south. The county covers an area of 44,175 square kilometers. Garissa County is low lying with altitudes ranging between 70 m and 400 m above sea level. The River Tana, which runs along the western boundary of the County, is the only permanent river. Though it is not confined within the county's boundaries, the river has tremendous influence over the climate, settlement patterns, and economic activities within the County, as it forms the single most important source of water for the fast growing Garissa Town and the surrounding areas. The county is generally semi-arid and receives annual rainfall of about 40 mm.

The soils range from the sandstone, dark clays in some patches, to alluvial soils along the river Tana basin. Garissa County's economy is mainly pastoral. The main economic activity is keeping of cattle, camels, goats and sheep. Increased livestock population has led to overgrazing and competition over watering grounds. The county receives rain in two seasons, these are the long rains season between March and April and the short rain season between October and December. The rainfall is unreliable with some torrential rains which in many cases are detrimental to vegetation growth. The temperatures in the county are high ranging from 20^{o}C to 38^{o}C. The natives of Garissa County are mainly Somali's whose main activity is pastoralism, and their staple food is mainly meat and milk (Arid lands development project, Kenya 2011). The study was focused in Garissa Township and Balambala sub-counties highlighted **(Figure 3.1).**

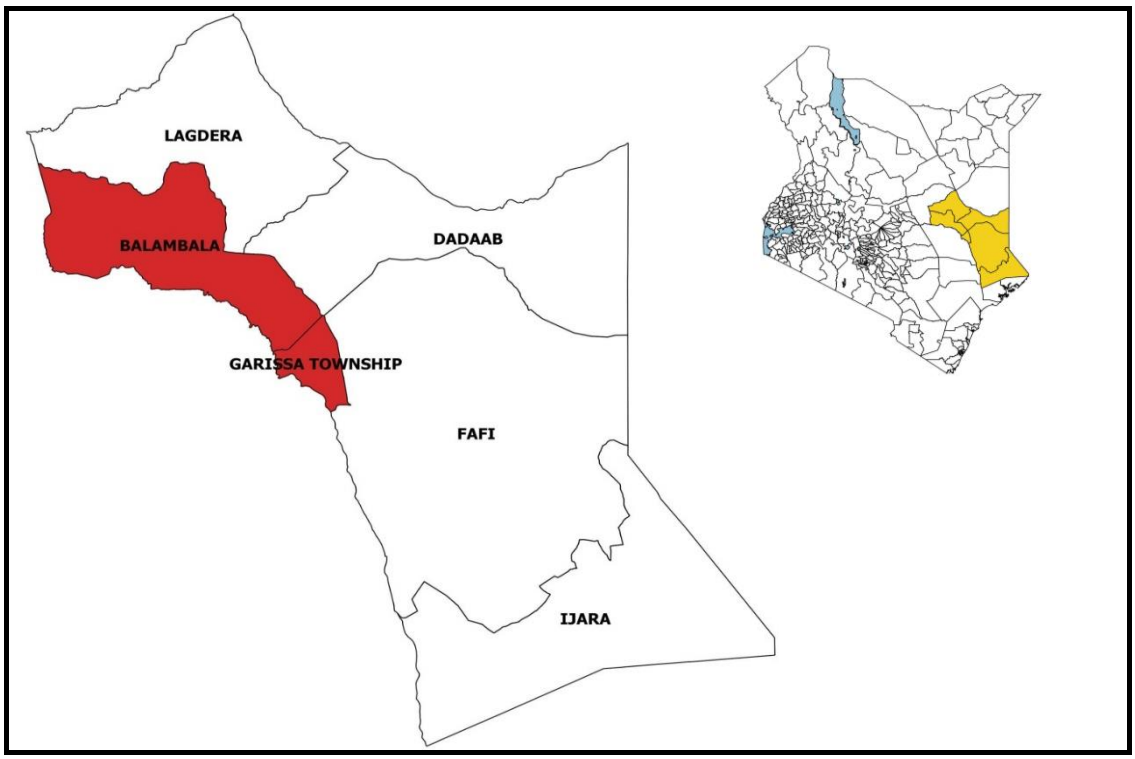

Figure 3.1: Map of Kenya showing Garissa County highlighted in yellow color and study area highlighted in red color

3.2 Study design

This was a cross-sectional study done in Garissa County, Balambala and Garissa Township sub-counties.

3.3 Study Population

Goat and sheep populations in the County comprised the study population. According to 2009 Kenya national housing and population census, the sheep and goat population in the county was estimated at 3, 315,061 sheep and goats with 2, 090,613 goats and 1,224,448 sheep. Garissa (constituting Balambala and Garissa Township) had estimated

goat population of 1,000,856 and 312,601 sheep and a total of 1,313,457 **(Table 3.1)**. The two sub-counties were chosen due to the large population of sheep and goats compared to other sub-counties. The pastoralists' within Balambala and Garissa sub-counties also constituted the study population

Table 3.1: Sheep and goats population, Garissa County

Sub-county	**Sheep**	**Goats**	**Total**
Garissa	312,601	1,000,856	**1,313,457**
Lagdera	489,282	561,883	**1,051,165**
Fafi	98,889	179,226	**278,115**
Ijara	323,676	348,648	**672,324**
Total	**1,224, 448**	**2,090,613**	**3,315,061**

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

3.4 Sample size determination for sheep and Goats

Herds were regarded as the primary sampling units. A herd was defined as any cluster or aggregate of animals, e.g. flock or group of animals under the same management system but not necessarily owned by a single individual that are able to frequently mix. Animals in a herd share common risk factors for disease, so the distribution of disease within the herd is assumed to be relatively homogenous. The total number of herds to be sampled was calculated using the formula for simple random sampling as suggested by (Thrushfield *et al.,* 2013).

Equation 1: Formula for Sample Size Calculations

$$n = \left(\frac{1.96}{\mathbf{d}}\right)^2 \times p(1-p) \qquad (1)$$

Where:

n= minimum number of herds or flocks to be sampled;

1.96= z-score at 95% confidence interval

d = desired absolute precision assumed at 10%;

p = expected herd or flock prevalence assumed at 16%

Based on a Brucellosis survey done in Kajiado, which has similar ecosystem as Garissa by the Kenya Zoonotic disease unit (ZDU), sero-prevalence of Brucellosis in goats was 16% and 11% in sheep (Osoro *et al.,* 2015). The sero-prevalence in goats was used to calculate the sample size. Based on the above parameters, a minimum herd sample size of 52 herds was determined. The resulting number of herds needed for sampling was multiplied by a design effect to consider the multistage level clustering of the sampling design that was employed. When no estimate of intra-cluster correlation coefficient is available, a qualitative assessment of intra-cluster correlation coefficient might be used (Ariwan and Frerichs, 1996). Suggested qualitative levels of ‘‘low,’’ ‘‘medium,’’ and ‘‘high’’ intra-cluster correlations are associated with values of the design effect: 2, 4, and 7, respectively were proposed. The design effect is the variance of an estimated proportion obtained by a cluster sample divided by the variance for a simple random sample (Dargatz and Hill, 1996). For this study, a low design effect of 2 was assumed due to high homogeneity and low heterogeneity within and between herds. The adjusted minimum sample size after multiplying with the design effect was 104 herds**.** In addition, 10% contingency was applied which gave rise to 114 minimum number of herds.

3.5 Sampling design for sheep and Goats

This was undertaken using a multiple stage sampling technique. The first stage was random selection of sub-locations in Garissa County to be sampled. As at the time of the study, sub-location were the lowest government administrative unit headed by assistant chief. Due to resource limitation a total of about a third of the sub-locations (12) was randomly selected from list of 33 sub-locations of Garissa County (**Table 3.2**).

Table 3.2: List of sub-locations in Garissa and Balambala Township and the Sub-locations randomly selected for Sampling highlighted in yellow

No	Sub-County	Name of sub-locations for sampling
1	Garissa Township sub-county	Township
2		Galbet
3		Medina
4		Malika
5		Waberi
6		Iftin
7		Korakora
8		Edet
9		Boralgi
10		Jarirot
11		Sankuri
12		Balich
13		Bololoweyn
14		Shidey
15		Raya
16		Atheley
17		Shabaha
18		Shimbirey
19		Abdisamit
20	Balambala sub-county	Saka
21		Saka junction
22		Daley
23		Balambala
24		Dujis
25		Jarajara
26		Kasha
27		Ashadin
28		Kuno

29		Danyere
30		Ohio
31		Kone
32		Sikley
33		Libahoi
34		Madey
35		Urgad
36		Dogob

Due to lack of comprehensive information on the number of sheep and goats herds (n) in the study area, a base line study was conducted. During the baseline study, the selected sub-locations were visited and with assistance of the local administration (assistant chief and community elders), local veterinary staff and community animal health workers (CAHWs), a comprehensive list of households and approximate number of animals owned by each household was generated. A final list of herds in a particular sub-location was generated putting into consideration the definition of herd as earlier outlined. Household(s) whose animals met criteria for a herd were merged and listed as a single herd under the name of the eldest member representing the merged households or as was agreeable among the merged households. This final list of households and number of animals owned by each household acted as a proxy to the number of herds of goats and sheep in each sub-location and constituted the sampling frame from which actual sampling was conducted. The second step was to identify the individual herds to sample in each sub-location. The number of herds to sample per sub-location depended on the number of herds in each sub-location (Sampling proportion to size). The third stage was a systematic random sampling (SRS) to select individual animals in the herd for sample collection. All eligible animals for sampling were placed within an enclosure with one gate. The first animal per species was randomly selected and subsequent animals were selected when animals of particular species were allowed to pass through the gate; factoring in the kth interval (sampling interval) determined based on the number of animals to be sampled within that species. This was repeated until the number of animals to be sampled for that species was achieved. Due to logistical reasons, sampling of individual animals within a herd was done proportional to size of the herd such that for

small herds (< 10 animals), all the animals in the herd were sampled, for medium herds with sizes (>10 but <50), all the animals to a maximum of 20 animals were sampled and for large herds, (>50 animals), a maximum of 20 animals were sampled. In this set up, sheep and goats are raised together as one herd therefore the above sample size applied to both. In any particular herd, sampling of goats and sheep was done proportional to their size within the herd. To facilitate sampling of the herds, list of selected herds for a particular sub-location were sent in advance to a community animal health worker (CAHW) within the sub-location to mobilize the pastoralists whose herds were selected for sampling. A total of 120 herds were sampled during the study and the distribution of the number of herds sampled per sub-location is as shown in the **Table 3.3** below.

Table 3.3: Distribution of number herds sampled per sub-location

Sub-location	Total herds listed	Number of herds sampled
Kuno	50	14
Madey	32	9
Shimbirey	42	12
Sikley	36	10
Balambala	54	15
Medina	30	8
Kasha	28	8
Dogob	18	5
Boralgi	38	11
Galbet	22	6
Ohio	44	12
Abdisamit	32	9
Total	**426**	**120**

3.6 Blood sample collection

Sheep and goats selected for sample collection were individually restrained and 9ml blood collected in plain red topped vacuum plastic tubes (Vacutainer®) from the jugular

vein. To allow clot separation, all blood samples were left to stand for approximately 15 to 30 minutes in a slanting manner at ambient temperature to separate serum from clot. Serum was collected from the Vacutainer using a disposable plastic Pasteur pipette, dispensed to an Eppendorf /cryovial tube and stored in a cool-box containing ice in the field. Eppendorf/cryovial tubes were then stored in the freezer at -20°C until used for serological testing.

3.7 Serological testing

The test procedures was done at the regional veterinary investigations laboratory (RVIL) in Garissa using test protocols as outlined by OIE (OIE, 2009a) and the manufacturer's specifications for the tests. Screening for presence of antibodies in collected sera specimens was done using Rose Bengal Plate test (RBPT) and complement fixation test (CFT). The sensitivity and specificity of RBPT were 89% and 97%, respectively and that of CFT was 88% and 100%. The CFT and RBPT test antigens (Brucella arbortus strain 99), control sera and other reagents were obtained from Atlas medical, William James House, Cowley Rd. Cambridge Cb4, 4WX and sensitized sheep red blood cells (SRBC) were obtained from the central veterinary laboratory (national veterinary reference laboratory in Kenya). The sera specimens were tested serially first using RBPT then CFT for those that tested positive on RBPT. An animal was considered positive if the serum specimen tested positive on both RBPT and CFT whereas a herd was considered positive if at least a single serum specimen from an animal within the herd tested positive on both RBPT and CFT. For RBPT, Rose Bengal test antigen was prepared from killed standard strain of B. abortus strain 99 and stained with Rose Bengal dye, in an acidic buffer pH 3.65. Serum samples and the antigen were left at room temperature for an hour before the test commenced. The bottle containing the antigen was well shaken so that the suspension was homogeneous. Thereafter 30µl of the sample was added after swirling for a minute onto a white tile and same volume of antigen alongside the antigen spots using a micro-pippete. The sera and antigen were then thoroughly mixed using a wooden splint, using one wooden splint for each test, until a

circular zone of approximately 2 cm was formed. The white tile was then rocked on both clockwise and anti-clockwise for four minutes (timing was done using a laboratory buzzer). Agglutination (due to antigen and antibody complex formation) was thereafter observed in a well-lit place to avoid false positive reading due to formation of fibrin. Magnifying glass was used to examine those tests that were suspected to have micro-agglutination. A positive test results was any visible agglutination observed and negative results was absence of any visible agglutination. Control serum was tested every day to provide minimal agglutination before the actual testing began to verify the sensitivity of the test conditions. For CFT, there was dilution of the test serum and appropriate working standards with equal volume of veronal buffered saline in small tubes which were then incubated at 58°C for 50 minutes to inactivate the native complement. Thereafter, 25µl of diluted test serum was dispensed on a round bottom 96 well micro-titre on the first and second rows of the well. Then 25µl of the veronal buffered saline was added to all wells except those on the first row. Serial doubling dilution was applied by transferring 25µl of serum from third row onwards and discarded 25µl of the mixture in last row. This serial dilution was done four times. This was followed by dispensing 25µl of antigen to each well except in the first row followed by 25µl of complement to each well. Control wells having diluent only; control wells having complement and diluent; and control wells having diluent, complement and antigen each at 75µl volume in each control wells were then set up. Control serum that results into a minimum positive reaction for each set of tests to ascertain the sensitivity of test conditions was tested each day. This was followed by incubation of the plates at 37°C for 30 minutes and thereafter addition of 25µl of sensitized sheep red blood cells (SRBC) to each well. This was followed by re-incubation of the plates at 37°C for 30 minutes. Thereafter the plates were centrifuged at 100rpm for 10 minutes to allow the SRBC that did not undergo hemolysis to settle. The degree of hemolysis was compared with standard corresponding to 0, 25, 50, 75 and 100% hemolysis. Absence of complementary activity was also checked for the serum in the first row. Sera samples having SRBCs sedimentation at a dilution $\geq$ 1:5 were considered to be positive for Brucellosis. Using

the diagnostic sensitivity and specificity of RBPT and CFT, combined sensitivity and specificity for the RBPT and CFT using a serial interpretation was calculated to be 78% and 100% respectively (Ausvet animal health services, 2009). This combined sensitivity and specificity together with the resulting apparent prevalence (AP) were factored in a formula suggested by Rogan and Gladen (Rogan and Gladen, 1978) to obtain the true prevalence (TP) at both the individual animal level and herd level.

Equation 2: Equation for determination of true sero-prevalence

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1} \qquad (2)$$

3.8 Data Management

3.8.1 Questionnaire survey

A pre-tested structured questionnaire was used to collect information about potential factors associated with Brucellosis herd sero-positivity and knowledge, attitude and practices of pastoralists towards Brucellosis. The potential factors included herd size, stocking density, management and breeding practices, purchasing of breeding stock in the past one year; keeping other animals with sheep and goats and history of abortion in animals in past one year. Some of the questions to assess knowledge attitude and practices towards Brucellosis included what is Brucellosis, causes in both humans and livestock, transmission, symptoms and signs in animals and humans, occurrence of disease in the farmer/ farmer's family/ neighbor, attitude towards handling infected animals, drinking raw milk and consumption of unprocessed milk products and practices like assisting animals during parturition or during abortion and methods of disposal of aborted fetuses and placenta. The questionnaire was administered immediately after bleeding the animals and services of a trained Somali speaking translator was used for ease of administration. The translator was trained on questionnaire administration prior to embarking on data collection to minimize on time taken to administer the questionnaires. The pre-testing of the questionnaire was done on five herds to check for consistency and any ambiguity. Subsequently, revisions were made on the questionnaire based on pre-testing findings.

3.8.2 Data analysis

Data was entered, cleaned and analyzed using Epi Info 7 (CDC, Atlanta, GA, USA) and Ms. Excel 2013 (Microsoft, Seattle, WA, USA). Univariate analysis was performed where proportions' was calculated for categorical variables and means and medians for continuous variables. Bivariate analysis was carried out to evaluate the association between herd Brucella sero-positivity and the potential factors. Odds ratios and 95% confidence interval (CI) was calculated and factors with p-value of ≤ 0.05 were considered statistically significant.

For selection of independent variables for inclusion into the initial multiple logistic regression model, the entry criterion was p-value ≤ 0.20. The model was developed by stepwise forward selection approach, dropping the least significant independent variable until all the remaining predictor variables were significant (p-value ≤ 0.05). All biologically and statistically plausible two-way interactions between variables remaining in the final model was tested and retained if significant.

3.9 Ethical Approvals and Considerations

Protocol approval was sought and obtained from Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and ethical clearance was sought and obtained from Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee (KNH-UoN ERC). The aim and procedures of the study was explained to participants' who were required to give written consent prior to their voluntary participation in the study. Blood samples were collected from animals of consenting individuals and were only used to test for antibodies against Brucellosis. Confidentiality of laboratory information was observed and maintained.

CHAPTER FOUR

RESULTS

4.1 Sero-prevalence of Brucellosis at Individual and Herd Level

4.1.1 Herd characteristics

A total of 2,400 animals composed of 979 (41%) sheep and 1471 (59%) goats were sampled. These animals were sampled from 120 herds from 12 out of the 36 sub-locations in Garissa Township and Balambala sub-counties. The 120 herds had a total of 12,945 animals of which 5,263 were sheep and 7,682 were goats. The median herd size was 112 animals (range: 58 to 140 animals).

4.1.2 Brucella Sero-prevalence at individual animal level by animal species

Of the 2,400 sera samples examined, 18.2% (95% CI: 16.7% to 19.8%) were positive for Brucella antibodies on RBPT. Of the samples positive on RBPT, 14.1%; (95% CI: 12.1% to 16.4%) were from sheep and 20.3% (95% CI: 18.4% to 22.5%) were from goats. Of the samples testing positive on RBPT, 16.4% (95% CI: 15.0% to 18.0%) tested positive on CFT of which 10.6% (95% CI: 8.8% to 12.7%) were sheep and 19.7% (95% CI: 17.8% to 21.8%) were goats (**Figure 4.1**).

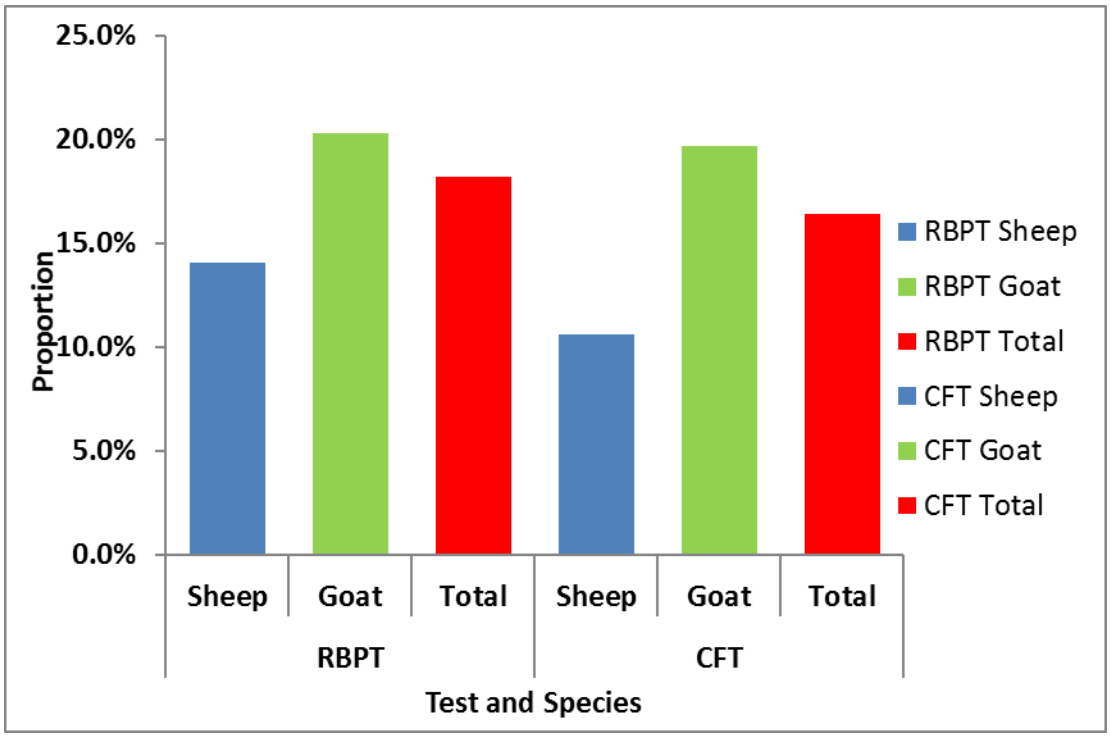

Figure 4.1: Distribution of Prevalence of Brucella antibodies by test type and species, Garissa County, 2013 (n=2,400)

4.1.3 Brucella Sero-prevalence at individual animal level by sub-location sampled

Based on CFT, highest sero-prevalence of 37.8% (95% CI: 30.92% to 45.03%) was recorded in Abdisimit sub-location followed by 25.4% (95% CI: 20.21% to 31.21%) in Ohio and 22.5% (95% CI: 17.9% to 27.67%) in Kuno; whereas the lowest sero-prevalence of 8.5% (95% CI: 5.2% to 13%) was recorded in Sikley and 2% (95% CI: 0.82% to 4.11%) in Balambala **(Table 4.1)**.

Table 4.1: Distribution of individual animal prevalence of Brucella antibodies in sheep and goats by sub-location, Garissa 2013 (n=2400)

Sub-location	Total number sampled	CFT Positive	95% CI
		n (%)	(Lower Limit to Upper Limit)
Abdisimit	180	68 (37.8)	30.9 – 45.0
Ohio	240	61 (25.4)	20.2 – 31.2
Kuno	280	63 (22.5)	17.9 – 27.7
Galbet	140	28 (20)	14.0 – 27.2
Madey	180	32 (17.8)	12.7 – 23.9
Shimbirey	240	39 (16.3)	12.0 – 21.3
Boralgi	220	33 (15.0)	10.7 – 20.2
Dogob	100	15 (15.0)	9.0 – 23.0
Kasha	160	17 (10.6)	6.5 – 16.1
Medina	160	15 (9.4)	5.6 – 14.7
Sikley	200	17 (8.5)	5.2 – 13.0
Balambala	300	6 (2)	0.8 – 4.1
Total	**2400**	**394 (16.4)**	**15.0 – 18.0**

4.1.4 Adjusted Individual animal level Sero-Prevalence

Considering samples that were positive by both the RBPT and CFT, and when adjusted to the two tests sensitivities and specificities, the overall true sero-prevalence of Brucellosis at individual animal level in sheep and goats was 20.0% (95% CI: 18.2% to 22.0%); and in goats it was 24.3% (95% CI: 21.8% to 27.1%) and sheep 12.5% (95% CI: 10.2% to 15.2%).

4.1.5 Brucella Sero-prevalence at Herd Level

At the herd level, of the 120 herds sampled, the overall apparent herd prevalence was 51.7% 95% CI: 42.8% to 60.4%). The overall true herd sero-prevalence of Brucellosis was 65.8% (95% CI: 54.3% to 77.2%); in goats it was 24.4% (95% CI: 18.2% to 22.0%) and in sheep it was 12.5% (95% CI: 10.2% to 15.2%). Among the herds with positive animals, the median number of animals testing positive was 8 animals (range: 3 to 15 animals). A total of 14 herds had four animals testing positive, 13 herds had five animals testing positive and 14 herds had 10 animals testing positive **(Table 4.2).**

Table 4.2: Comparison of Number of Herds with Positive Reactors with Number of animals testing Positive (n=62)

Number of animals testing positive	Number of herds with positive reactors
Less than 3	0
3	9
4	14
5	13
6	2
7	1
8	5
9	1
10	14
11	1
12	1
13	0
14	0
15	1
Above 15	0
Total	**62**

4.2 Factors Associated with Brucellosis Herd Sero-positivity

4.2.1 Bivariate Analysis for Herd level Factors Associated with Brucellosis in sheep and goats

In determining both the risk factors and protective factors associated with Brucella herd sero-positivity, several factors were examined at the bivariate analysis **(Table 4.3).** Since Garissa County is in a pastoral set up where herds mix especially during grazing and at watering points, 98 (82%) of the herds had come into contact with other herds of sheep and goats. Herds that came into contact with other herds had almost 4 times higher odds of having positive reactors to for Brucella compared to those herds that did not come into contact with other herds and this was statistically significant (OR= 3.6; p-value = 0.02). In contrast, coming into contact with wildlife or wildlife abortus was not associated with herd Brucella sero-positivity.

In overall, abortion in sheep and goats herds was reported in 84 (70%) of the herds in past one year. Of the herds that tested sero-positive, 52 (84%) had experienced abortion in the past year compared to 32 (55%) in sero-negative herds. A herd that experienced abortions within the past year were more than 4 times increased odds to have sero-positive animals compared to herds that had not experienced abortion, an association that was statistically significant (OR = 4.2; p-value = 0.001). However, experiencing abortion in cattle herds (OR= 0.3; p-value = 0.52) was not associated with herd Brucella sero-positivity in sheep and goats.

When comparing herds that introduced a new animal into the herd in the past year to those herds that did not, herds that introduced new animals into the herds were more than eight times increased chances of having sero-positive reactors for Brucellosis compared to those that did not (OR =8.1; p-value <0.001).

The chances of a herd having seropositive reactors was almost three times higher among those herds that lend male animals to other herds for breeding purposes as compared to those herds that did not lend their male animals to other herds for breeding. This was also statistically significant (OR= 2.8; p-value = 0.04).

Herds whose owners sought veterinary services in the past year compared to those that whose owners did not seek veterinary services in the past year, had 60% reduced chances of having sero-positive reactors to Brucella and this was statistically significant (OR= 0.4; p-value = 0.02). Herd size (OR= 1.6; p-value = 0.33) was not statistically associated with Brucella herd sero-positivity in sheep and goats.

All the respondents' did not have calving pens for the herds, none of the animals introduced into the herd over past year were quarantined before introduction, all the respondents' did not vaccinate their animals against Brucellosis.

Table 4.3: Comparison of factors associated with Brucella Sero-positivity among positive and negative herds, Garissa 2013

(Positive herds n=62; Negative herds n=58)

Variable	Herds Positive n (%)	Herds Negative n (%)	POR* (95% CI)	P-value
Contact other sheep and goats herds in past year				
Yes	56 (90)	42 (72)	3.6 (1.3 - 9.9)	0.02
No	6 (10)	16 (28)	Reference	
Contact wildlife in past year				
Yes	23 (37)	17 (29)	1.4 (0.7 - 3.1)	0.48
No	39 (63)	41 (71)	Reference	
Contact wildlife abortus in past year				
Yes	10 (44)	6 (35)	1.4 (0.4 - 5.1)	0.85
No	13 (56)	11 (65)	Reference	
Type of wildlife contact in past year				
Ruminant	15 (65)	8 (47)	2.1 (0.6 – 7.6)	0.41
Non-ruminant	8 (35)	9 (53)	Reference	
Abortion in sheep and goat herd in past year				
Yes	52 (84)	32 (55)	4.2 (1.8 - 9.9)	0.001
No	10 (16)	26 (45)	Reference	
Abortion in cattle herd in past year				
Yes	1 (50)	4 (80)	0.3 (0.01 - 8.6)	0.52
No	1 (50)	1 (20)	Reference	
Introduction of new animals into sheep and goat herd in past year				
Yes	37 (60)	9 (16)	8.1 (3.4 – 19.3)	<0.001
No	25 (40)	49 (84)	Reference	
Lend male animals to other sheep and goat herds for breeding in past year				
Yes	21 (34)	9 (16)	2.8 (1.2 - 6.8)	0.04
No	41 (66)	49 (84)	Reference	
Seek veterinary services in past year				
Yes	15 (24)	27 (47)	0.4 (0.2 - 0.8)	0.02
No	47 (47)	31 (53)	Reference	
Herd size				
<100	21 (34)	14 (24)	1.6 (0.7 – 3.6)	0.33

***POR=Prevalence odds ratio**

≥100	41 (64)	44 (76)	Reference

4.2.2 Multivariable analysis to determine independent factors associated with Brucella herd sero-positivity

The multivariable logistic regression model revealed that introduction of new animals into the herd [adjusted odd ratio (aOR) = 8.0; p-value <0.0001), experiencing abortion in sheep and goats herd in past year (aOR = 3.4; 95%; p-value=0.01) and seeking of veterinary services (aOR = 0.3; 95% CI: 0.1 to 0.8) were the independent factors associated with sheep and goats herds sero-positivity to *Brucella* antigens, with introduction of new animals into the herd and experiencing abortion in sheep and goats herds in past year being risk factors and seeking veterinary services/advice in past year being protective factors **(Table 4.4).**

Table 4.4: Multivariable Logistic Regression Analysis of the Variables associated with Herd-level Sero-positivity for *Brucella* spps in Sheep and Goats, Garissa County, 2013

(Positive herds n=62; Negative herds n=58)

Variable Characteristics	Odds Ratio**	95% CI	Coefficient	S.E.	Z-Statistic	P-Value
Introduction of new animals into sheep and goats herd in past year (Yes/No)	8.0	3.1- 20.7	2.08	0.486	4.28	<0.0001
Experience abortion in sheep and goats herds in past year (Yes/No)	3.4	1.3 – 8.9	1.23	0.485	2.54	0.01
Seeking veterinary advice/services in past year (Yes/No)	0.3	0.1 – 0.8	-1.20	0.471	-2.55	0.01

**aOR=adjusted odds ratio

4.3 Determination of Knowledge, Attitude and Practices towards Brucellosis among pastoralists in Garissa County

4.3.1 Demographic characteristics of study participants

A total of 120 participants were interviewed to assess their knowledge, attitude and practices towards Brucellosis. The median age of the study participants was 16 years (Range: 15 – 70 years), with 102 (85%) aged below 35 years. There were 90 (75%) males; and 92 (77%) of all study participants had no formal education. In regard to primary role of the study participants in management of the herd, 58 (48%) were herd owners, 38 (32%) were herders and 24 (20%) were mostly involved in milking animals. Eighty-three (69%) of the participants were married and 37 (31%) were single **(Table 4.5).**

Table 4.5: Distribution of Socio-demographic characteristics of study participants, Garissa County-2013 (n=120)

Variable	Frequency n (%)
Age group	
15-24	53 (44)
25-34	49 (41)
35-44	14 (12)
>45	4 (3)
Gender	
Male	90 (75)
Female	30 (25)
Education level	
No formal education	92 (77)
At least primary education	28 (23)
Primary role in Herd	
Herd owner	58 (48)
Herding	38 (32)
Milking	24 (20)
Marital status	
Married	83 (69)

Single	37 (31)

4.3.2 Knowledge of pastoralists toward Brucellosis

4.3.2.1 Awareness and cause of Brucellosis in animals and Humans

Among the study participants, 95 (79%) had heard of Brucellosis and the local name for Brucellosis in Somali was "Diis". Among those who had heard of Brucellosis, 17 (18%) mentioned germs/bacteria as the cause of Brucellosis in both animals and humans **(Figure 4.3).** Of the source of information about Brucellosis, 44 (46%) heard of it through community health workers, 19 (20%) from a family member, 19 (20%) religious leaders, eight (8%) from veterinary staff and five (5%) from local FM stations.

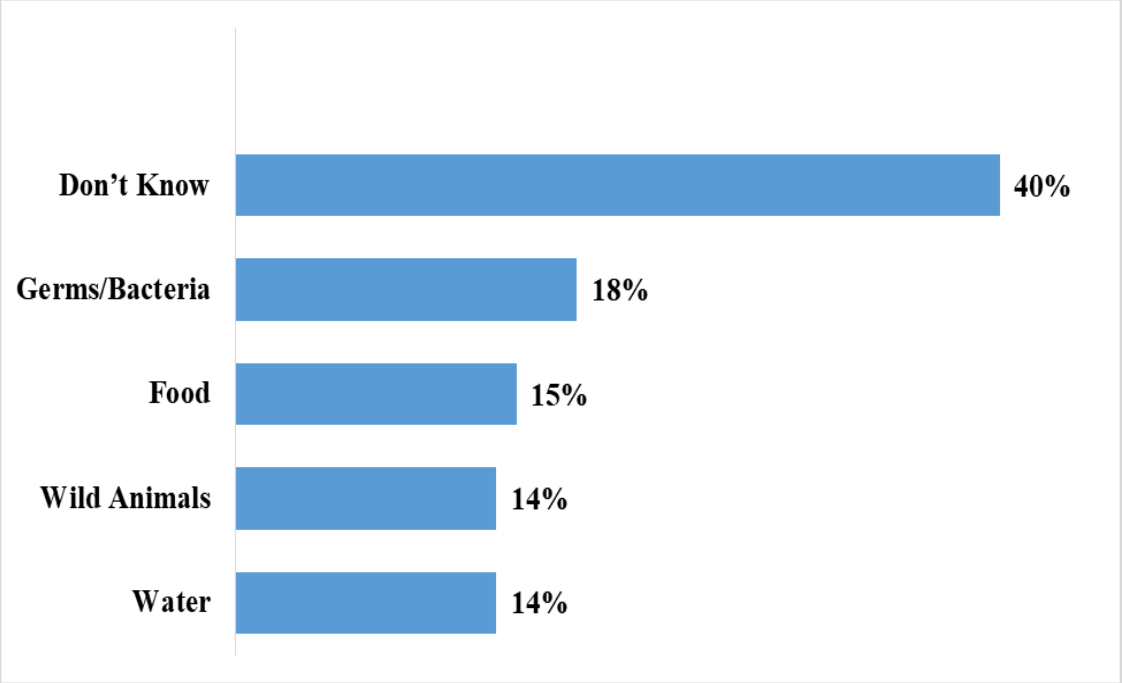

Figure 4.2: Distribution of study participants' responses on causes of Brucellosis, Garissa County, 2013 (n=95)

4.3.2.2 Knowledge of respondents'about the animals affected and signs/symptoms of Brucellosis in animals

In regard to animal species affected by Brucellosis, 62 (65%) mentioned goats, 47 (49%) camels, 45 (47%) cattle and 32 (34%) sheep. Fifty-six (59%) of respondents mentioned abortion as most common sign, 21 (22%) mentioned retained placenta, 20 (21%) swollen joints or hygroma and 11 (12%) mentioned mastitis or swollen udder and teats **(Table 4.6).**

Table 4.6: Distribution of responses of participants' on animal species affected by Brucellosis and signs and symptoms of Brucellosis in animals (n=95)

	Frequency
Variable	**n (%)**
Animal species affected	
Goats	62 (65)
Camels	47 (49)
Cattle	45 (47)
Sheep	32 (34)
Signs and symptoms of Brucellosis**	
Abortions	56 (59)
Retained Placenta	21 (22)
Swollen joints	20 (21)
Mastitis	11 (12)

**** Respondents were allowed to choose multiple responses**

4.3.2.3 Knowledge of respondents on modes of transmission of Brucellosis to humans

A summary of information about the knowledge of respondents about mode of transmission of Brucellosis to humans is in **Figure 4.3.** Concerning Brucellosis being zoonotic disease, only 27 (28%) of the respondents knew of this. Among the respondents who knew that Brucellosis is a zoonotic disease, 22 (82%) mentioned drinking raw milk as most common mode of transmission of Brucellosis from animals to humans, followed by eating milk products from raw milk mentioned by 11 (41%) respondents.

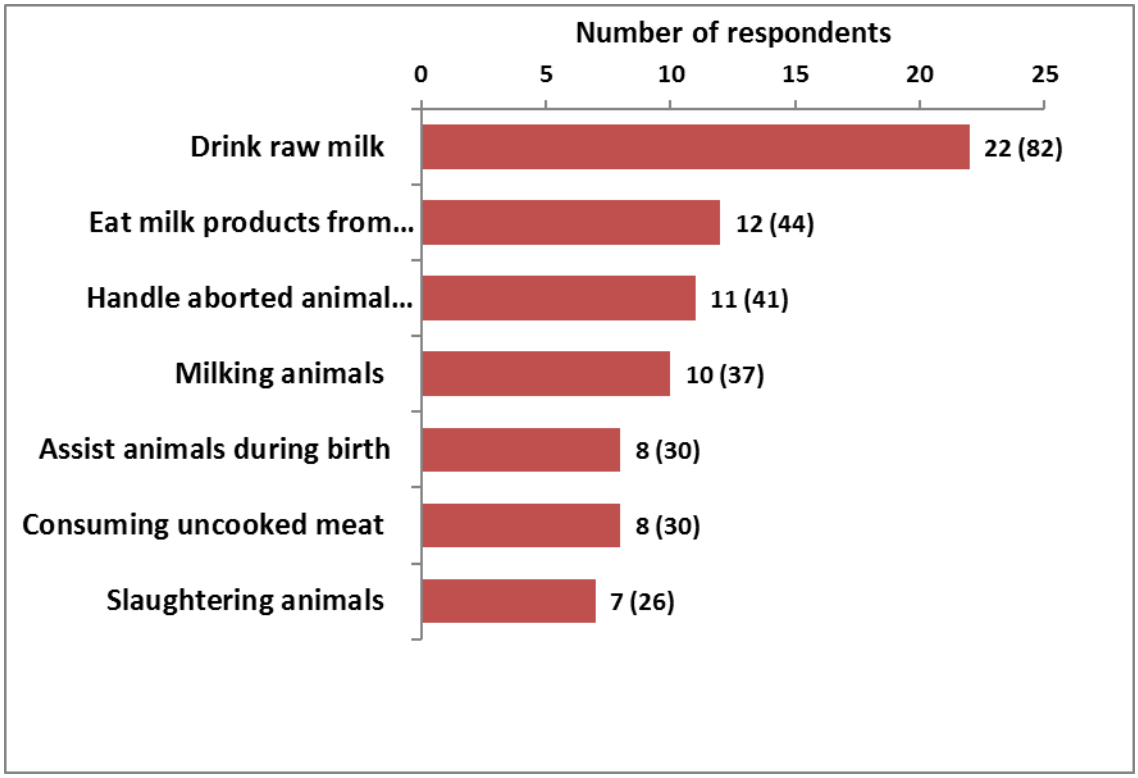

Figure 4.3: Distribution of respondents' responses on mode of transmission of Brucellosis to humans, Garissa County, 2013 (n=27)

4.3.2.4 Knowledge of respondents on signs/symptoms of Brucellosis in humans

Summary of information on the knowledge of respondents' on signs and symptoms of Brucellosis in humans is as shown in **Table 4.7.** Forty-six (48%) of the respondents had a family member diagnosed with Brucellosis in the past, 43 (45%) had seen somebody who is not a family member with Brucellosis and 38 (40%) respondents had been diagnosed with Brucellosis in the past. Concerning signs and symptoms, 71 (75%) of respondents mentioned fever, 45 (47%) chills, 37 (47%) night sweats, 14 (15%) painful scrotum in men and 7 (8%) spontaneous abortion in women.

Variable	Frequency n (%)
Brucellosis occurrence	
Family member diagnosed with Brucellosis in the past	46 (48)
Seen person not family diagnosed with Brucellosis	43 (45)
Self was diagnosed with Brucellosis in the past	38 (40)
Signs and symptoms	
Fever	71 (75)
Loss of appetite	56 (59)
Joint pain	48 (51)
Chills	45 (47)
Headache	38 (40)
Night sweats	37 (39)
Back pain	33 (35)
Fatigue	29 (31)
Malaise	29 (31)
Vomiting	14 (15)
Painful scrotum in men	14 (15)
Diarrhea	12 (13)
Blurred vision	9 (10)
Miscarriage in women	7 (8)
Nausea	5 (5)

Table 4.7: Responses of respondents' on signs and symptoms of Brucellosis in humans, Garissa County, 2013 (n=95)

4.3.3 Respondents' attitude towards Brucellosis

A summary of information about the attitude of study participants about Brucellosis is summarized in **Table 4.8**. A total of 63 (67%) of the respondents thought that Brucellosis is a serious disease in animals whereas 61 (64%) thought that Brucellosis is a serious disease in human. Only 13 (14%) thought that Brucellosis can be prevented in animals of which six (46%) mentioned vaccination. In regard to attitude and perception of respondents when they suspect they have Brucellosis, 33 (35%) thought that Brucellosis in human can be treated/cured of which 14 (42%) mentioned visiting health facility. When encountered with an aborting animal in the herd, 83 (87%) of the respondents would do nothing.

Table 4.8: Distribution of respondents' responses on attitude and perceptions towards Brucellosis, Garissa County, 2013 (n=95)

Attitudes and perceptions	Frequency n (%)
Attitude and Perception on Brucellosis seriousness	
Serious Disease in Animals	64 (67)
Serious Disease in Humans	61 (64)
Attitude towards Brucellosis prevention in animals	
Brucellosis can be prevented in animals	13 (14)
Prevention by vaccination	6 (46)
Prevention by contacting veterinary office	4 (31)
Prevention by isolation of sick and aborting animals	3 (23)
Attitude when suspecting human Brucellosis	
Brucellosis can be cured in humans	33 (35)
Visit health facility	14 (42)
Praying	8 (24)
Consuming herbal medicine	6 (18)
Visit local chemist and purchase medicine	5 (15)
Attitude towards aborting animals	
Do nothing	44 (46)
Treat aborting animals with antibiotics	16 (17)
Sell	11 (12)
Inform veterinary office	10 (11)
Isolate	8 (8)
Slaughter	5 (5)

4.3.4 Respondents' practices towards Brucellosis

Respondents' responses regarding practices towards Brucellosis are as shown in **Table 4.9.** A total of 91 (96%) of the respondents consumed raw milk in the past year and 34 (36%) assisted an animal during parturition process, abortion or removal of retained placenta and none used gloves during the process. All (100%) disposed fetal material by dumping.

Table 4.9: Distribution of respondents' responses on practices towards Brucellosis, Garissa County, 2013 (n=95)

Practices of respondents	**Frequency n (%)**
Consumption of raw milk	91 (96)
Participation in slaughtering/butchering an animal	72 (76)
Assisted an animal during birthing/abortion/removal of retained placenta	34 (36)
Used gloves when assisting during abortions and parturitions	0 (0)
Disposal of fetal materials	
Dumping	34 (100)
Burning	5 (15)
Burying	0 (0)
Consumption of raw milk products	13 (14)

4.3.5 Respondents' requirement of more Information on Brucellosis

A total of 92 (97%) of the respondents believed that they were not sufficiently informed about Brucellosis and required more information. The most favored mode of receiving information on Brucellosis was through the local FM radio stations mentioned by 36 (39%) of respondents, 23 (25%) favored religious leaders, 18 (20%) local community meetings (barazas) and 15 (16%) community health workers/community animal health workers.

CHAPTER FIVE

DISCUSSIONS, CONCLUSSION AND RECOMMEDATIONS

5.1 Discussion

5.1.1 Highlight of major findings of the study

This study reports the serological analysis of exposure to *Brucella* spps in sheep and goats and determination of factors associated with sero-positivity from a population based sample of sheep and goats herds in Garissa district (currently Garissa Township and Balambala sub-counties). The study estimated the true sero-prevalence of Brucellosis in sheep and goats both at the individual animal and herd level incorporating the sensitivity and specificity of the diagnostic tests used and the uncertainties in these tests. The result of this study does confirm that there is very high level of probability that the sheep and goats herds in Garissa could have been exposed to *Brucella* spps hence enhanced chances of zoonotic transmission to humans and exposure to other livestock species like cattle and camels which are also susceptible to Brucellosis and are also reared in this setup. The study also highlights the need for adjusting for the sensitivity and specificity of serological tests used in order to make reliable interpretation of serological surveys, since lack of adjustment based on sensitivity and specificity could lead to unbiased estimates of sero-prevalence resulting into significantly different estimates. Seroprevalence of Brucellosis in sheep and goats in this set up varied by abortion status of the herd and introduction of new animals into the herd which were risk factors and seeking veterinary care which was protective factor. The study also examined the farmers' knowledge, attitudes and practices towards Brucellosis and highlights the knowledge gaps that exist among pastoralists in the study area towards Brucellosis as well as the poor attitude and practices that put them at risk of getting infected with Brucellosis.

5.1.2 Sero-prevalence of Brucellosis in sheep and goats

Adjusted Brucellosis sero-prevalence in the sheep and goats herds in Garissa County appears to be high at 20.0% individual animal level and 65.8% at herd-level. This prevalence varied between the sheep and goats. Reports from literature suggest a very variable small ruminant Brucellosis sero-prevalence at individual and herd-level in Kenya across various African countries. Estimates include animal-level sero-prevalence of between 0.4% and 9.7%, and 15% to 45% herd-level sero-prevalence in Ethiopia (Megersa *et al*., 2011; Mohammed *et al*., 2015; Tsehay *et al*., 2014; Tsegay *et al*., 2015; Dabassa *et al*., 2013; Yesuf *et al*., 2012; Deddefo *et al*., 2015). In Niger, between 0.4% and 3.6% individual animal-level and 17.8% herd-level sero-prevalence have been found (Boukary *et al*., 2013); In Egypt between 0.44% and 12.1% individual animal-level (Hegazy *et al*., 2011a and Hegagazy *et al*., 2011b); In Nigeria individual animal level of 3.3% in sheep and 4.5% in goats (Brisibe *et al*., 1996) have been reported; In Sudan between 0.9% and 22% individual animal-level (Gumaa *et al*., 2014; Abdallah *et al*., 2015; Omer *et al*., 2007) and in Uganda, 4% individual animal-level and 13% herd-level sero-prevalence in goats have been reported (Kabagambe *et al*., 2001). In Kenya, studies done in Kiambu, Kajiado and Marsabit Counties estimated individual animal-level sero-prevalence ranging from 1.3% to 16.1% and herd-level sero-prevalence ranging from 5.6% to 68% (Osoro *et al*., 2015). Another study done in Baringo County estimated an individual animal sero-prevalence of 13.04% in goats and 8.23% in sheep (Kosgei *et al*., 2015). These variations in estimates of sero-prevalence of Brucellosis in small ruminants may be largely attributed to the varied animal husbandry and production systems, various agro-ecological zones in which the studies were carried out, the sampling methodology employed and diagnostic tests. These factors have been shown to contribute to variation in the obtained sero-prevalence of Brucellosis infection in livestock among different researchers (Mangen *et al*., 2002; McDermott and Arimi, 2002; Nielsen, 2002; Díaz-Aparicio *et al*., 2002). Another important issue is the difference in sensitivity and specificity of serological tests used for screening. This

factor contributes to the variability of results among researchers (Nielsen, 2002). The RBPT used for screening individual animals in this study is a cheap, rapid and highly sensitive test (OIE 2009a). However, its specificity is low because the smooth lipopolysaccharides of the Brucella antigen can cross-react with antibodies from closely related Gram-negative bacteria such as Yersinia enterocolitica, Escherichia coli, *Salmonella* spps and Sternotrophomonas maltophilia as well as antibodies produced by *B. abortus* S19 vaccine (Nielsen, 2002). The study also found varied differences in sero-prevalence of Brucellosis between sheep and goats. This could be attributed to the greater susceptibility of goats to Brucella infection and also excreting the organism for longer periods compared to the sheep (Tsehay, *et al*., 2014).

5.1.3 Herd-level factors associated with Brucellosis sero-positivity in sheep and goats

The introduction of new animals into the sheep and goats herd from unscreened herds was a major risk factor observed in this study. These animals are usually introduced into the herd as replacement stocks by the pastoralists. This result is in agreement with several authors who found out that the introduction of animals from non-free Brucellosis herds or from herds of which their Brucellosis status was unknown was a major factor associated with Brucellosis both in sheep and goat herds and in cattle (Kabagambe *et al*., 2001, Refai, 2002; Coelho *et al*., 2008; Bamaiyi *et al*., 2014; Sharifi *et al*., 2012; Sharifi *et al*., 2014). Other studies suggest that the introduction of infected animals can lead to an increase in the individual level prevalence due to the fact that the longer they are in contact with rest of the flock, the higher the risk of spread would be (Corbel, 2006; Rahman *et al*., 2013). Practices that involve movement of animals between herds have also been found likely to be risky. Evidence exists that one of the main reasons for the inefficiency of most Brucellosis control campaign is the lack of control in the movement of animals and one suggested key prevention measure is to avoid introduction of infected animals by maintaining a completely closed herd or by carefully screening

purchased animals before introducing them into the herd, a practice that is very rare in pastoral communities (Kabagambe *et al*., 2001, Refai, 2002; Nielsen *et al*., 1990).

Presence of female animals which had aborted in the herd was found to be significantly associated with herd sero-positivity. Abortion in livestock represents the major complaint attributed to Brucella infections (Kabagambe *et al*., 2001; Muma *et al*., 2007a; Muma *et al*., 2007b, Musa *et al*., 2008; Schelling *et al*., 2003). Females infected with Brucellosis are known to excrete high concentrations of the organism in their milk, placental membranes and aborted fetuses. Such females have been reported to continue shedding the organisms for several months (Radostits *et al*., 2007). This results into environmental contamination and as a result there is enhanced high risk of transmission of the pathogen between animals of the same herd as well as other herds during free mixing in grazing and watering places. Zoonotic transmission to humans through direct contact with contaminated material such as fetal membranes and aborted fetuses is also enhanced. This is supported by the fact that majority of the respondents' interviewed reported assisting animals during abortions and birthing processes and handling fetal materials without any protective clothing putting them at risk of coming down with the disease should such materials be contaminated with Brucella organisms. Similarly the owners reported consuming unpasteurized milk and milk products and just dumping aborted fetuses and fetal membranes, practices which clearly shows their ignorance of the transmision of the disease (Obonyo & Gufu; 2015).

Lending male animals to other herds for breeding purposes and seeking veterinary services in the past year were also factors that were significantly associated with Brucellosis herd sero-positivity with the former being a risk factor and the latter a protective factor. Lending of male animals for breeding has been reported by other authors to be a risk factor associated with Brucella sero-positivity in animals (Al-Majali *et al*., 2007; Reviriego *et al.,* 2000). Although venereal route is not considered an important route for Brucellosis transmission in small ruminants under natural conditions,

practices that involve movement of animals between flocks are considered risky due to potential of mechanical transmission (Radostits *et al*., 2007; Benkirane, 2006).

Though not significant in the final multivariable model, contact between herds was found as a significant risk factor at the bivariate analysis. This could be attributed to the nomadic pastoral lifestyle of the community where there is frequent migration of pastoral herds. Considering the contagious nature of *Brucella* spps, sharing common grazing land and drinking water places facilitate transmission of Brucellosis as well as other diseases between potentially infected herds and clean herds (Megersa *et al*., 2011; Schelling *et al*., 2003; Smits, 2014).

5.1.4 Knowledge Attitude and Practices towards Brucellosis

The KAPs survey showed that Brucellosis is known by the general community in the present study area, since more than three quarters of the study respondents had heard of Brucellosis. This is similar to findings of previous studies done in Uganda among pastoral communities living along Lake Mburo; in Egypt among cattle and Buffalo farmers in a village in Nile Delta region and among small ruminant farmers in the peri-urban areas of Dushanbe Tajikistan in which 99.3%, 83.2% and 57% of the respondents' had heard of Brucellosis (Kansiime *et al*., 2014; Holt *et al*., 2013; Grahn, 2013).

However, the awareness of Brucellosis among study participants in Uganda and Egypt were higher compared to our study but that in Tajikistan was lower. In contrast to this finding, a study done among small-scale dairy farmers in an urban and peri-urban area of Tajikistan and another one done among urban and peri-urban dairy and non-dairy farming households in Kenya found that most respondents had not heard of Brucellosis. In the Kenyan study done in Kiambu County, 30% of dairy respondents and 22% of non-dairy respondents knew of the existence of Brucellosis whereas in Tajikistan 85% of the respondents had never heard of Brucellosis (Lindahl *et al*., 2015; Kange'the *et al*., 2007). Perhaps an explanation as to why the pastoral communities are more aware of Brucellosis compared to farmers in urban or peri-urban areas could be due to their close proximity and interaction with animals resulting into in-built indigenous knowledge over years which is subsequently passed down from one generation to the next. Despite a

higher proportion of the study participants had heard about Brucellosis, majority had little or no knowledge about the cause of the disease. Less than a fifth of the participants correctly mentioned germ/bacteria as cause of Brucellosis. Poor knowledge regarding etiology of Brucellosis could negatively impact on respondents' preventive and control methods of Brucellosis in both humans and animals due to misconception on the cause hence need to enhance public health education in this set up.

The main sources of information on Brucellosis in this study area was community health workers (CHWs) followed by family members. Contrary to this finding, the study in Uganda and the two studies in Tajikistan found main source of information to be from friends/relatives (Kansiime *et al*., 2014; Grahn, 2013; Lindahl *et al*., 2015). Few participants in the current study mentioned mass media (radio/TV) as a source of information about Brucellosis, which was similar to the studies in Uganda and Tajikistan. These findings implies the powerful role the community health volunteers play in terms of relaying important health messages to nomadic pastoralists in this area who in most circumstances have challenges in accessing basic health care services. Deliberate moves should therefore be undertaken to incorporate the two in all aspects of health care education for the pastoralists.

Based on results of this study, the respondents' had basic knowledge about the animal species affected and signs/symptoms of Brucellosis in animals. In this regard, about two thirds mentioned goats, close to a half sheep and cattle, and majority were not aware that camels could be affected. This findings contrast with the findings of studies in Tajikistan (Lindahl *et al*., 2015) where 82% of respondents knew that cattle, sheep and goats could be affected and the study in Egypt (Holt *et al*., 2011) in which 98.1% mentioned cattle, 86% sheep and 85% goats. However, the current study was fairly in agreement with another study in Tajikistan (Grahn, 2013) in which two thirds mentioned that all animals could be affected. With regards to clinical signs of Brucellosis in animals, more than half of the respondents mentioned abortion as the major clinical sign. This finding was in agreement with findings of a study done among pastoralists in Kaduna state in Nigeria

and the study in Egypt in which 94.4% and 59.5% of respondents mentioned abortion as the major clinical sign (Holt *et al*., 2011; Buhari *et al*., 2015). However the current study finding was different from that done in Tajikistan where only 11% of respondents' mentioned abortion as a clinical signs of Brucellosis in animals (Grahn, 2013). Knowledge of the animal species affected and signs/symptoms of Brucellosis in animals are crucial because it positively impacts on farmers' practices towards prevention and control measures of Brucellosis in both animals and humans.

In this study, majority of the study participants did not know that Brucellosis is a zoonotic disease, findings which were similar to those in previous studies conducted in Ghana and Nigeria which found very low awareness of zoonotic nature of Brucellosis (Buhari *et al*., 2015; Addo *et al*; 2011). Among those who were aware of the zoonotic nature of Brucellosis, consumption of raw milk and raw milk products was the most cited mode of transmission of Brucellosis from animals to humans. The respondents' response regarding consumption of milk as a mode of transmission was comparable to findings in Egypt and Uganda (Kansiime *et al*., 2014; Holt *et al*., 2011). However in the current study, the respondents' had low awareness on other modes of transmission such as handling of aborted fetuses and fetal membranes, consumption of raw or undercooked meat, assisting animals during parturition and slaughtering animals; most of which have been identified in many studies as major risk factors for transmission of Brucellosis from animals to humans (Kozukeev *et al*., 2003; Earhart *et al*., 2009). Such low knowledge on mode of transmission of Brucellosis from animals to humans have been documented elsewhere (Grahn, 2013; Buhari *et al*., 2015; Addo *et al*., 2011).

In the current study, the majority of the study participants identified fever, joint pains and muscle pains in that order as the major signs and symptoms of Brucellosis. This was consistent with the findings of a previous study in Kyrgyzstan where fever and joint pain (locally known as "Tajik") were mentioned as main signs and symptoms of Brucellosis in humans (Grahn, 2013) as well as a study in Nigeria where all respondents knew signs and symptoms of Brucellosis in humans (Adesokan *et al*., 2013). However, the finding

of the current study is different from the results of previous studies conducted in other parts of Nigeria and in Ghana (Buhari *et al*., 2015; Addo *et al*., 2011) where almost all participants were not aware of signs and symptoms of Brucellosis in humans. The respondents' basic knowledge about the signs and symptoms of Brucellosis in humans could have significant impact if the community knowledge is enhanced thus reducing diagnosis and treatment delay which in the long run will prevent sequelae and prolonged human suffering.

The present study showed that a considerable proportion of the study respondents perceived that Brucellosis was a serious disease in both animals and humans. However, despite this high perception of risk, most respondents' had poor attitudes towards prevention of Brucellosis in animals and treatment of Brucellosis in suspected humans. Regarding respondents' opinion on actions most would take when confronted with an aborting animal in their herd, majority would do nothing about it whereas others would attempt treating the animal with antibiotics or sell the animal. Very few mentioned isolation of the animal or seeking veterinary services. Failure to isolate suspected animals has been cited as one of the major risk factors for transmission of Brucellosis within and between herds as susceptible animals can become infected through contact with infected animals aborted tissues or consumption of pasture or water contaminated with aborted materials (Laing and Wagner, 1988). Frequent migration of pastoralists with their animals increases the chances of different herds coming into contact with other potentially infected herds thus spreading diseases (Megersa *et al*., 2011; Boukary *et al., 2013*). This is more important when considering the high levels of infectiousness of Brucellosis making practices such as sharing grazing land and drinking water points by pastoral communities a major transmission pathway of Brucellosis between different herds (Makita *et al*., 2011; Mekonnen *et al*., 2010).

The study participants engaged in risky practices that could expose them to infection with Brucellosis. Nearly all respondents consumed raw milk, about three quarter assisted animals during abortions or parturition and handled aborted materials/fetal membranes

and a third participated in slaughtering or butchering an animal. Of those who assisted aborting animals, three quarter dumped the aborted materials and none used any protective clothing. Such risky practices have been shown to be important risk factors for Brucellosis transmission to human (Kozukeev *et al*., 2003; Earhart *et al* 2009; Sofian *et al*., 2008; Kiambi, 2014; Regassa *et al*., 2009). Female animals infected with *Brucella* spps excrete high concentrations of the organism in their milk, placental membranes and aborted fetuses (Radostits *et al*., 2007; Laing and Wagner, 1988). Goats have also been shown to have prolonged secretion of Brucella organisms in milk compared to sheep (Poester & Santos, 2013). Furthermore, *Brucella* spps have been shown to survive in aborted fetuses, manure and water for periods of up to 150 to 240 days (Saegerman *et al*., 2010). Therefore, there is a high risk of transmission of the pathogen between animals and from animals to humans through direct contact with contaminated materials such as fetal membranes, aborted fetuses, manure and other animal products. Introduction of new animals into the herd without quarantine and borrowing or lending breeding males to other farmers or even taking a female to be served at a neighbor's farm have been identified as major risk factors for transmission of Brucellosis within and between herds as shown in studies in several countries (Boukary *et al*., 2013; Kabagambe *et al*., 2001; Muma *et al*., 2007b, Al-Majali *et al*., 2009; Chand & Chhabra, 2013, Chiebao *et al*., 2013; Patel *et al*., 2014).

5.2 Limitations of the study

1. Study focused on small ruminants' Brucellosis only and left out other livestock species: cattle and camels deemed to be susceptible to Brucellosis. The study also did not study Brucellosis in humans to correlate findings in the animals.
2. The present study did not attempt culture of the Brucella organisms and therefore was not able to identify the various species and biovars of Brucella orgaisms circulating in the sheep and goats
3. The present was also not able to evaluate the socio-economic impact of the disease in humans and in limiting livestock production in the study area.

5.3 Conclusions and Recommendations

5.3.1 Conclusions

1. The present study confirms a considerably high prevalence of Brucellosis both at individual animal and herd level in sheep and goats. Therefore there is enhanced potential for zoonotic transmission and exposure to other susceptible livestock species that are reared in the region.
2. The introduction of new non-quarantined animals with unknown Brucellosis status into the herd, occurrence of abortions within the herd and lending of male animals for breeding purposes were found to be the major risk factors for the spread of the disease within and between herds.
3. In addition, the study showed that the community had some knowledge towards Brucellosis but attitude and practices were poor. At present, there is no officially coordinated program for control of Brucellosis in Kenya. Understanding of the knowledge, perceptions and practices have been defined as important pillars regarding the feasibility and the acceptability of potential measures that might be instituted. Enhanced public health education on the cause, symptoms and mode of transmission of Brucellosis would be important towards the prevention and control of Brucellosis in the present study area. This can be achieved by targeted messages in local FM radios and integrating the community health volunteers in control and prevention efforts.

5.3.2 Recommendations

1. Necessary measures should be taken by the national and County governments involved in health of livestock and humans in the region to prevent the transmission of Brucellosis to the human population and as well as transmission of Brucellosis to other livestock species. There is therefore need for enhanced public health education as a prevention and control strategy for Brucellosis in both humans and animals in this region.

2. Due to nomadic pastoral lifestyle of the community, control of Brucellosis in livestock is quite challenging not only because of the number and complexity of risk factors involved, but also because the risk factors that are tightly linked and often inherent to the livestock production practices. The above factors when combined with the close interaction between the people and their livestock coupled with the socio-cultural lifestyle and poor knowledge attitude and practices poses a serious public health concern and has been known to enhance transmission of Brucellosis to the human population. These reasons make control of Brucellosis both in livestock and humans challenging. At present, there is no officially coordinated program for control of Brucellosis in Kenya. However the greater horn of Africa where Kenya belongs has made greater strides towards controlling of the disease. The African Union Inter-African Bureau on Animal Resources (AU-IBAR) came up with a strategy dubbed "Standard Methods and Procedures (SMPs) for control of Brucellosis in the Greater Horn of Africa" which outlines minimum standards, methods and procedures on various subject areas of surveillance, laboratory procedures and disease control that must be met for harmonized regional control of the disease (AU-IBAR, 2014). If fully adopted, these SMPs will go a long way towards control of livestock Brucellosis in the greater horn of Africa. However for the full potential of the SMPs to be realized, there is need to evaluate attitudes of communities involved and their roles need to be clearly defined in the various control strategies especially regarding the feasibility and the acceptability of potential measures. Measures including selective vaccination programs combined with the slaughtering of known infected animals (test and slaughter) as well as testing animals newly introduced into the herd can be considered. This suggested control measures can only be effective with involvement and buy –in of the community including integrating the CAHWs in the control strategies.

3. Other studies to be conducted in the region should include other species of livestock which are also susceptible to Brucellosis as well as humans in a linked animal-human study; attempt culture of the Brucella organisms to identify the various species and biovars of *Brucella* spps circulating in both livestock and human in this region and evaluate the socio-economic impact of the disease in humans and iit effect on limiting livestock production.
4. However for effective control of Brucellosis in the present study area, an integrated approach should be promoted that takes into account the relationship between humans, animals and environment in the context of ‘‘One health approach’’.

REFERENCES

Abdallah, A. A., Elfadil, A. A. M., Elsanosi, E. M., and Shuaib, Y. A. (2015). Sero-prevalence and Risk Factors of Brucellosis in Sheep in North Kordofan State. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), 8*(1), 31-39.

Acha N.P., and Szyfres B. (2003). *Zoonoses and Communicable Diseases Common to Man and Animals*, third ed., (vol. 1.) Washington: DC *Pan American Health Organization* (PAHO).

Addo, K. K., Mensah, G. I., Nartey, N., Nipah, G. K., Mensah, D., Aning, G. A., and Smits, H. L. (2011). Knowledge, Attitudes and Practices (KAP) of herdsmen in Ghana with respect to milk-borne zoonotic diseases and the safe handling of milk. *Journal of Basic and Applied Scientific Research, 1*(10), 1566-1562.

Adesokan, H. K., Alabi, P. I., Stack, J. A., and Cadmus, S. I. (2013). Knowledge and practices related to bovine Brucellosis transmission amongst livestock workers in Yewa, south-western Nigeria. *Journal of the South African Veterinary Association, 84*(1), 1-5.

Alton, G. G., Jones, L. M., Angus, R. D., and Verger, J. M. (1988). Techniques for the Brucellosis laboratory. *Institut National de la recherche Agronomique (INRA), Paris, France*, pp. 63–129.

Alton G.G. (1990). Brucella melitensis. In: Nielsen, K., Duncan, J.R. (Eds.), Animal Brucellosis., pp. 383–410. Boca Raton, FL: CRC Press, Boca Raton, FL

Al-Majali, A. M. (2005). Seroepidemiology of caprine Brucellosis in Jordan. *Small Ruminant Research, 58*(1), 13-18.

Al-Majali, A. M., Majok, A. A., Amarin, N. M., and Al-Rawashdeh, O. F. (2007). Prevalence of, and risk factors for, Brucellosis in Awassi sheep in Southern Jordan. *Small Ruminant Research*, *73*(1), 300-303.

Al-Majali, A. M., Talafha, A. Q., Ababneh, M. M., and Ababneh, M. M. (2009). Seroprevalence and risk factors for bovine Brucellosis in Jordan. *Journal of Veterinary Science*, *10*(1), 61-65.

Arid lands development project, Kenya (2005). Garissa County vision and strategy 2005-2015 Retrieved from: *www.aridland.go.ke/NRM_Strategy/garissa.pdf*

Ariwan, I., and Frerichs R.R. (1996). *User's Manual: CSurvey, Version 1.5. University of Indonesia*, , Los Angeles: Indonesia and University of California.

Ashagrie, T., Deneke, Y., and Tolosa, T. (2011). Sero-prevalence of caprine Brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia. *African Journal of Microbiology Research*, *5*(13), 1682-1685.

AU-IBAR (2014). Standard Methods and Procedures (SMPs) for Control of Brucellosis in the Greater Horn of Africa. Nairobi. Retrieved from: www.au-ibar.org/component/jdownloads/finish/76-tmt/2116-standard-methods-and-procedures-smps-for-control-of-Brucellosis-in-the-greater-horn-of-africa

Australian veterinary animal health services (2009). Epi-tools. Retrieved from: *www.ausvet.com/epitools*

Baddour, M. M., and Alkhalifa, D. H. (2008). Evaluation of three polymerase chain reaction techniques for detection of Brucella DNA in peripheral human blood. *Canadian journal of microbiology*, *54*(5), 352-357.

Bamaiyi, P. H., Hassan, L., Khairani-Bejo, S., ZainalAbidin, M., Ramlan, M., Krishnan, N., and Hashim, S. N. (2014). Case–control study on risk factors associated with Brucella Melitensis in goat farms in Peninsular Malaysia. *Tropical animal health and production*, *46*(5), 739-745.

Bekele, M., Mohammed, H., Tefera, M., and Tolosa, T. (2011). Small ruminant Brucellosis and community perception in Jijiga County, Somali Regional State, eastern Ethiopia. *Tropical Animal Health and Production*, *43*(4), 893-898.

Benkirane, A. (2006). Ovine and caprine Brucellosis: World distribution and control/eradication strategies in West Asia/North Africa region. *Small Ruminant Research, 62*(1), 19-25.

Bishop G.C., Bosman P.P., and Herr S., (1994). *Bovine Brucellosis. In: Infectious diseases of livestock. Eds. Coetzer, Thomson and Tustin*, (Volume 2: 1053-1066), Capetown/ R.S.A.: Oxford University Press.

Blasco, J. M. (1997). A review of the use of B. melitensis Rev 1 vaccine in adult sheep and goats. *Preventive veterinary medicine*, *31*(3), 275-283.

Boukary, A. R., Saegerman, C., Abatih, E., Fretin, D., Bada, R. A., De Deken, R., Harouna, H.A., Yenikoye, A., and Thys, E. (2013). Seroprevalence and potential risk factors for Brucella spp. infection in traditional cattle, sheep and goats reared in urban, periurban and rural areas of Niger. *PloS one*, *8*(12), e83175.

Brisibe, F., Nawathe, D. R., and Bot, C. J. (1996). Sheep and goat Brucellosis in Borno and Yobe States of arid northeastern Nigeria. *Small Ruminant Research*, *20*(1), 83-88.

Buhari, H. U., Saidu, S. N. A., Mohammed, G., and Raji, M. A. (2015). Knowledge, attitude and practices of pastoralists on bovine Brucellosis in the north senatorial County of Kaduna state, Nigeria. *Journal of Animal Health and Production*, *3*(2), 28-34.

Carrera, I. A., Rodríguez, M. J. L., Sapiña, A. M., Lafuente, A. L., and Sacristán, A. R. B. (2006). Probable transmission of Brucellosis by breast milk. *Journal of tropical pediatrics*, *52*(5), 380-381.

Chand, P., and Chhabra, R. (2013). Herd and individual animal prevalence of bovine Brucellosis with associated risk factors on dairy farms in Haryana and Punjab in India. *Tropical Animal Health and Production*, *45*(6), 1313.

Chiebao, D. P., Valadas S. Y. O. B., Minervino A. H. H., Castro V., Romaldini A. H. C. N., Calhau A. S., … and Soares R. M. (2015). Variables Associated with Infections of Cattle by Brucella abortus Leptospira spp. and Neospora spp. in Amazon Region in Brazil. *Transboundary and emerging diseases*, *62*(5), e30-e36.

Colmenero, J. D., Queipo-Ortuño, M. I., Reguera, J. M., Suarez-Muñoz, M. A., Martín-Carballino, S., and Morata, P. (2002). Chronic hepatosplenic abscesses in Brucellosis. Clinico-therapeutic features and molecular diagnostic approach. *Diagnostic microbiology and infectious disease*, *42*(3), 159-167.

Coelho, A. M., Coelho, A. C., Góis, J., de Lurdes Pinto, M., and Rodrigues, J. (2008). Multifactorial correspondence analysis of risk factors for sheep and goat Brucellosis sero-prevalence. *Small Ruminant Research*, *78*(1), 181-185.

Coelho, A. M., Coelho, A. C., and Rodrigues, J. (2013). Seroprevalence of sheep and goat brucellosis in the northeast of Portugal. *Archivos de Medicina Veterinaria*, *45*(2).

Corbel, M. J. (1997). Brucellosis: an overview. *Emerging infectious diseases*, *3*(2), 213.

Corbel M.J., Banai M., Genus I., Brucella M. and Shaw 1920, 173AL. (2005). In: Brenner DJ, Krieg NR, Staley JT, editors. Bergey's manual of systematic bacteriology. vol. 2. New York: Springer; p. 370-86.

Corbel, M. J. (2006). *Brucellosis in humans and animals*. Geneva: World Health Organization.

Center for Food security and Public Health report (2009). Ovine and Caprine Brucellosis: Brucella melitensis. Retrieved from: http://www.cfsph.iastate.edu/Factsheets/pdfs/Brucellosis_melitensis.pdf

Cutler, S. J., Whatmore, A. M., and Commander, N. J. (2005). Brucellosis–new aspects of an old disease. *Journal of applied microbiology*, *98*(6), 1270-1281.

Dabassa, G., Tefera, M., and Addis, M. (2013). Small ruminant Brucellosis: Serological survey in Yabello County, Ethiopia. *Asian Journal of Animal Science*, *7*, 14-21.

Dargatz, D. A., and Hill, G. W. (1996). Analysis of survey data. *Preventive Veterinary Medicine*, *28*(4), 225-237.

Deddefo, A., Sisay, T., and Tuli, G. (2015). Sero-prevalence and risk factors of small ruminant Brucellosis in selected Countys of Arsi and East Shoa zones, Oromia region, Ethiopia. *African Journal of Microbiology Research*, *9*(19), 1338-1344.

Díaz-Aparicio, E., Marin, C., Alonso-Urmeneta, B., Aragón, V., Pérez-Ortiz, S., Pardo, M., and Moriyon, I. (1994). Evaluation of serological tests for diagnosis of

Brucella melitensis infection of goats. *Journal of clinical microbiology*, *32*(5), 1159-1165.

Earhart, K., Vafakolov, S., Yarmohamedova, N., Michael, A., Tjaden, J., and Soliman, A. (2009). Risk factors for Brucellosis in Samarqand Oblast, Uzbekistan. *International Journal of Infectious Diseases*, *13*(6), 749-753.

Farrell I. D. (1974). The development of a new selective medium for the isolation of *Brucella abortus from* contaminated sources. *Research in Veterinary Science,* 16, 280-286.

Ferede, Y., Mengesha, D., and Mekonen, G. (2011). Study on the seroprevalence of small ruminant Brucellosis in and around Bahir Dar, North West Ethiopia. *Ethiopian Veterinary Journal*, *15*(2).

Foster, G., Osterman, B. S., Godfroid, J., Jacques, I., and Cloeckaert, A. (2007). Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. *International journal of systematic and evolutionary microbiology*, *57*(11), 2688-2693.

Foster, J. T., Beckstrom-Sternberg, S. M., Pearson, T., Beckstrom-Sternberg, J. S., Chain, P. S., Roberto, F. F., Hnath, J., Brettin, T., and Keim, P. (2009). Whole-genome-based phylogeny and divergence of the genus Brucella. *Journal of bacteriology*, *191*(8), 2864-2870.

Fretin D., Fauconnier A., Kohler S., Halling S., Leonard S., Nijskens C., Ferooz J., Lestrate P., Delrue R.M., Danese I., Vandenhaute J., Tibor A., DeBolle X. and Letesson, J.J. (2005). The sheathed flagellum of Brucella melitensis is involved in persistence in a murine model of infection. *Cell Microbiology*. *7*, 687–698.

Fretin, D., Fauconnier, A., Köhler, S., Halling, S., Léonard, S., Nijskens, C., … and Vandenhaute, J. (2005). The sheathed flagellum of Brucella melitensis is involved in persistence in a murine model of infection. *Cellular microbiology*, *7*(5), 687-698.

Fugier, E., Pappas, G., and Gorvel, J. P. (2007). Virulence factors in Brucellosis: implications for aetiopathogenesis and treatment. *Expert Reviews in Molecular Medicine*, *9*(35), 1-10.

Garissa County (2013). Garissa County Profile. Retrieved from: www.garissa.go.ke.

Godfroid J., Brucella melitensis infection (2004). *Infectious Diseases in Livestock.* (Volume 3. 2nd edition). Edited by Coetzer JAW, Tustin RC. Cape Town: Oxford University Press.

Godfroid, J., Cloeckaert, A., Liautard, J. P., Kohler, S., Fretin, D., Walravens, K., Garin-Bastuji, B., and Letesson, J. J. (2005). From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, Brucellosis has continuously been a re-emerging zoonosis. *Veterinary research*, *36*(3), 313-326.

Godfroid, J., Nielsen, K., and Saegerman, C. (2010). Diagnosis of Brucellosis in livestock and wildlife. *Croatian medical journal*, *51*(4), 296-305.

Grahn, C. (2013). Brucellosis in small ruminants.

Gumaa, M. M., Osman, H. M., Omer, M. M., El Sanousi, E. M., Godfroid, J., and Ahmed, A. M. (2014). Seroprevalence of Brucellosis in sheep and isolation of Brucella abortus biovar 6 in Kassala State, Eastern Sudan. *Revue scientifique et technique (International Office of Epizootics)*, *33*, 957-65.

Halling, S.M. (2005). Completion of the genome sequence of *Brucella abortus* and comparison to the highly similar genomes of *Brucella melitensis* and *Brucella suis*. *Journal of Bacteriology*., *187*(8), .2715-2726.

Halling, S. M., Peterson-Burch, B. D., Bricker, B. J., Zuerner, R. L., Qing, Z., Li, L. L., Kapur, V., Alt, D.P., and Olsen, S. C. (2005). Completion of the genome sequence of Brucella abortus and comparison to the highly similar genomes of Brucella melitensis and Brucella suis. *Journal of Bacteriology*, *187*(8), 2715-2726.

Hegazy, Y. M., Molina-Flores, B., Shafik, H., Ridler, A. L., and Guitian, F. J. (2011a). Ruminant Brucellosis in Upper Egypt (2005–2008). *Preventive veterinary medicine*, *101*(3), 173-181.

Hegazy, Y. M., Moawad, A., Osman, S., Ridler, A., and Guitian, J. (2011b). Ruminant Brucellosis in the Kafr El Sheikh Governorate of the Nile Delta, Egypt: prevalence of a neglected zoonosis. *PLoS Neglected Tropical Diseases*, *5*(1), e944.

Holt, H. R., Eltholth, M. M., Hegazy, Y. M., El-Tras, W. F., Tayel, A. A., and Guitian, J. (2011). Brucella spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating sero-prevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). *BMC Public Health*, *11*(1), 1.

International Committee on Systematics of Prokaryotes (ICSP) (2010). Sub-committee on the Taxonomy of *Brucella.* Retrieved from: http://www.the-icsp.org/subcoms/Brucella.htm.

Kabagambe, E. K., Elzer, P. H., Geaghan, J. P., Opuda-Asibo, J., Scholl, D. T., and Miller, J. E. (2001). Risk factors for Brucella seropositivity in goat herds in eastern and western Uganda. *Preventive Veterinary Medicine*, *52*(2), 91-108.

Kato Y., Masuda G., Itoda I., Imamura A., Ajisawa A. and Negishi M. (2007). Brucellosis in a returned traveler and his wife: probable person-to person transmission of Brucella melitensis. *Journal of Travel Medicine*. *14*, 343–345.

Kang'ethe, E. K., Ekuttan, C. E., Kimani, V. N., and Kiragu, M. W. (2007). Investigations into the prevalence of bovine Brucellosis and the risk factors that predispose humans to infection among urban dairy and non-dairy farming households in Dagoretti Division, Nairobi, Kenya. *East African medical journal*, *84*(11 Supplement), S96-100.

Kansiime, C., Mugisha, A., Makumbi, F., Mugisha, S., Rwego, I. B., Sempa, J., and Rutebemberwa, E. (2014). Knowledge and perceptions of Brucellosis in the pastoral communities adjacent to Lake Mburo National Park, Uganda. *BMC public health*, *14*(1), 1.

Kenya Department of Veterinary Services (1999-2010). *Annual reports. Ministry of Livestock Development Kenya*. Nairobi: KDVS.

Khan, M. Y., Mah, M. W., and Memish, Z. A. (2001). Brucellosis in pregnant women. *Clinical infectious diseases*, *32*(8), 1172-1177.

Kiambi, S. G. (2014). *Prevalence and factors associated with Brucellosis among febrile patients attending Ijara County Hospital, Kenya* (Msc dissertation).

Kosgei, P. K., Bebora, L., Waiboci, L., Kitala, P., & Kiambi, S. (2015). Estimating prevalence of Brucellosis in livestock and assessment of knowledge,

attitudes and practices of respective communities in Baringo county, Kenya. In RUFORUM Fourth Biennial Conference, Maputo, Mozambique, 19-25 July 2014 (pp. 297-301). RUFORUM.

Kozukeev, T. B., Ajeilat, S., Maes, E., Favorov, M., and Centers for Disease Control and Prevention (CDC). (2006). Risk factors for Brucellosis--Leylek and Kadamjay Countys, Batken Oblast, Kyrgyzstan, January-November, 2003. *MMWR Morbidity Mortality Weekly Report*, *55*(Supplement 1), 31-34.

Laing, J. A., Morgan, W. J., and Wagner, W. C. (1988). Brucellosis In: Fertility and infertility in veterinary practice. *English book language book society, Bailliere, Tindall*, 189-220.

Lindahl, E., Sattorov, N., Boqvist, S., and Magnusson, U. (2015). A study of knowledge, attitudes and practices relating to Brucellosis among small-scale dairy farmers in an urban and peri-urban area of Tajikistan. *PloS one*, *10*(2), e0117318.

Makita, K., Fèvre, E. M., Waiswa, C., Eisler, M. C., Thrusfield, M., and Welburn, S. C. (2011). Herd prevalence of bovine Brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. *BMC veterinary research*, *7*(1), 60.

Mangen, M. J., Otte, J., Pfeiffer, D., and Chilonda, P. (2002). *Bovine Brucellosis in sub-Saharan Africa: estimation of sero-prevalence and impact on meat and milk offtake potential.* Rome: FAO.

Mantur, B. G., Amarnath, S. K., and Shinde, R. S. (2007). Review of clinical and laboratory features of human Brucellosis. *Indian journal of medical microbiology*, *25*(3), 188.

Marin, C. M., Alabart, J. L., and Blasco, J. M. (1996a). Effect of antibiotics contained in two Brucella selective media on growth of Brucella abortus, B. melitensis, and B. ovis. *Journal of Clinical Microbiology*, *34*(2), 426-428.

Marín, C. M., Jimenez de Bagüés, M. P., Barberán, M., and Blasco, J. M. (1996b). Comparison of two selective media for the isolation of Brucella melitensis from naturally infected sheep and goats. *Veterinary record*, *138*, 409-410.

McDermott, J. J., and Arimi, S. M. (2002). Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary microbiology*, *90*(1), 111-134.

Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J., and Skjerve, E. (2011). Sero-prevalence of Brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Tropical animal health and production*, *43*(3), 651-656.

Mekonnen, H., Kalayou, S., and Kyule, M. (2010). Serological survey of bovine Brucellosis in barka and arado breeds (Bos indicus) of Western Tigray, Ethiopia. *Preventive Veterinary Medicine*, *94*(1), 28-35.

Mikolon, A. B., Gardner, I. A., De Anda, J. H., and Hietala, S. K. (1998). Risk factors for brucellosis seropositivity of goat herds in the Mexicali Valley of Baja California, Mexico. *Preventive veterinary medicine*, *37*(1-4), 185-195.

Mohammed, S., Tuli, G., Nigatu, S., and Alemaw, G. (2015). Sero-Prevalence of Brucellosis in Goats Purchased for Slaughter in Selected Export Abattoirs, Ethiopia.

Moreno, E., Cloeckaert, A., and Moriyón, I. (2002). Brucella evolution and taxonomy. *Veterinary microbiology*, *90*(1-4), 209-227.

Muma, J. B., Godfroid, J., Samui, K. L., and Skjerve, E. (2007a). The role of Brucella infection in abortions among traditional cattle reared in proximity to wildlife on the Kafue flats of Zambia. *Revue scientifique et technique (International Office of Epizootics)*, *26*(3), 721-730.

Muma, J. B., Samui, K. L., Oloya, J., Munyeme, M., and Skjerve, E. (2007b). Risk factors for Brucellosis in indigenous cattle reared in livestock–wildlife interface areas of Zambia. *Preventive Veterinary Medicine*, *80*(4), 306-317.

Musa, M. T., Eisa, M. Z. M., El Sanousi, E. M., Wahab, M. A., and Perrett, L. (2008). Brucellosis in camels (Camelus dromedarius) in Darfur, Western Sudan. *Journal of comparative pathology*, *138*(2), 151-155.

Munoz, P. M., Marin, C. M., Monreal, D., Gonzalez, D., Garin-Bastuji, B., Diaz, R., Mainar-Jaime, R.C., Moriyon, I., and Blasco, J. M. (2005). Efficacy of several serological tests and antigens for diagnosis of bovine Brucellosis in the presence of false-positive serological results due to Yersinia enterocolitica O: 9. *Clinical and Diagnostic Laboratory Immunology*, *12*(1), 141-151.

Negash, E., Shimelis, S., and Beyene, D. (2012). Sero-prevalence of small ruminant Brucellosis and its public health awareness in selected sites of Dire Dawa region, Eastern Ethiopia. *Journal of Veterinary Medicine and Animal Health*, *4*(4), 61-66.

Nielsen, K., and Duncan, J. R. (1990). *Animal Brucellosis*. London: CRC press.

Nielsen, K. (2002). Diagnosis of Brucellosis by serology. *Veterinary microbiology*, *90*(1), 447-459.

Nielsen, K., Smith, P., Yu, W., Nicoletti, P., Jungersen, G., Stack, J., and Godfroid, J. (2006). Serological discrimination by indirect enzyme immunoassay between the antibody response to Brucella sp. and Yersinia enterocolitica O: 9 in cattle and pigs. *Veterinary immunology and immunopathology*, *109*(1), 69-78.

Nicoletti P. (2002). A short history of Brucellosis. *Veterinary. Microbiol*ogy, *90*(1-4), 5-9.

Obonyo, M., and Gufu, W. B. (2015). Knowledge, Attitude and Practices towards Brucellosis among Pastoral Community in Kenya, 2013. *International Journal of Innovative Research and Development*, *4*(10).

Office International des Epizooties (OIE) "Terrestrial manual, Bovine Brucellosis," (2009a). In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE, Paris, France, chapter 2.4.3

Office International des Epizooties (OIE), Caprine and Ovine Brucellosis (excluding Brucella ovis), (2009b). In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE, Paris, France, chapter 2.7.2.

Omer, M. K., Skjerve, E., Holstad, G., Woldehiwet, Z., and Macmillan, A. P. (2000). Prevalence of antibodies to Brucella spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems. *Epidemiology and Infection*, *125*(02), 447-453

Omer, M. M., Abdelaziz, A. A., Abusalab, S. M., and Ahmed, A. M. (2007). Survey of Brucellosis among sheep, goats, camels and cattle in Kassala area, Eastern Sudan. *Journal of Animal and Veterinary Advances*, *3*.

Osoro, E. M., Munyua, P., Omulo, S., Ogola, E., Ade, F., Mbatha, P., M. … and Guerra M. (2015). Strong Association Between Human and Animal Brucella Seropositivity in a Linked Study in Kenya, 2012–2013. *The American journal of tropical medicine and hygiene*, *93*(2), 224-231.

Ouahrani-Bettache, S., Soubrier, M. P., and Liautard, J. P. (1996). IS6501-anchored PCR for the detection and identification of Brucella species and strains. *Journal of applied bacteriology*, *81*(2), 154-160.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., and Tsianos, E. V. (2006). The new global map of human Brucellosis. *The Lancet infectious diseases*, *6*(2), 91-99.

Patel, M. D., Patel, P. R., Prajapati, M. G., Kanani, A. N., Tyagi, K. K., and Fulsoundar, A. B. (2014). Prevalence and risk factor's analysis of bovine Brucellosis in peri-urban areas under intensive system of production in Gujarat, India. *World*, *7*(7), 509-516.

Poester, F. P., Samartino, L. E., and Santos, R. L. (2013). Pathogenesis and pathobiology of Brucellosis in livestock. *Revue Scientifique Et Technique*, *32*, 105-115.

Queipo-Ortuño, M. I., Morata, P., Ocon, P., Manchado, P., and Colmenero, J. D. D. (1997). Rapid diagnosis of human Brucellosis by peripheral-blood PCR assay. *Journal of clinical microbiology*, *35*(11), 2927-2930.

Quinn P.J., Carter M.E., Markl B., and Carter G.R. (1999). Clinical Veterinary Microbiology by Mosby, Edinburgh pp. 261-267.

Radostits O.M., Blood D.C., and Gay, C.C. (2007). Veterinary Medicine, Text Book of Cattle, Horses, Sheep, Pig and Goats, 11th edition, 2007. London: WB Saunders Company Ltd., Philadelphia

Rahman, A. A., Saegerman, C., Berkvens, D., Fretin, D., Gani, M. O., Ershaduzzaman, M., Muzahed U. A., and Emmanuel, A. (2013). Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal Test and Slow Agglutination Test for the diagnosis of Brucellosis in sheep and goats in Bangladesh. *Preventive Veterinary Medicine*, *110*(2), 242-252.

Refai, M. (2002). Incidence and control of Brucellosis in the Near East region. *Veterinary microbiology*, *90*(1), 81-110.

Regassa, G., Desalew, M., Lawrence, Y., Hiwot, T., Teshome, G., Asfawesen, G., Abraham, A., Theresia, H.A., and Henk, L. S. (2009). Human Brucellosis in traditional pastoral communities in Ethiopia. *International Journal of Tropical Medicine*, 4(2), 59-64.

Reguera, J. M., Alarcon, A., Miralles, F., Pachon, J., Juarez, C., and Colmenero, J. D. (2003). Brucella endocarditis: clinical, diagnostic, and therapeutic approach. *European Journal of Clinical Microbiology and Infectious Diseases*, *22*(11), 647-650.

Reviriego, F. J., Moreno, M. A., and Domınguez, L. (2000). Risk factors for Brucellosis sero-prevalence of sheep and goat flocks in Spain. *Preventive Veterinary Medicine*, *44*(3), 167-173.

Rogan and Gladen (1978). Estimating prevalence from the results of a screening test. *American Journal of Epidemiology*, 107:71-76.

Saegerman, C., Berkvens, D., Godfroid, J., and Walravens, K. (2010). Bovine Brucellosis. *Infectious and parasitic disease of livestock*. p. 971-1001.

Samadi, A., Ababneh, M., Giadinis, N. D., and Lafi, S. Q. (2010). Ovine and caprine brucellosis (Brucella melitensis) in aborted animals in Jordanian sheep and goat flocks. *Veterinary medicine international*, *2010*.

Schelling, E., Diguimbaye, C., Daoud, S., Nicolet, J., Boerlin, P., Tanner, M., and Zinsstag, J. (2003). Brucellosis and Q-fever sero-prevalences of nomadic pastoralists and their livestock in Chad. *Preventive veterinary medicine*, *61*(4), 279-293.

Schlabritz-Loutsevitch, N. E., Whatmore, A. M., Quance, C. R., Koylass, M. S., Cummins, L. B., Dick Jr, E. J., Snider, C.L., Cappelli, D., Ebersole, J.L., Nathanielsz, P.W., and Hubbard, G. B. (2009). A novel Brucella isolate in association with two cases of stillbirth in non-human primates–first report. *Journal of medical primatology*, *38*(1), 70-73.

Scholz, H. C., Hubalek, Z., Sedláček, I., Vergnaud, G., Tomaso, H., Al Dahouk, S., S., Melzer, F., Kämpfer, P., Neubauer, H., Cloeckaert, A., and Maquart, M. (2008). Brucella microti sp. nov., isolated from the common vole Microtus arvalis. *International Journal of Systematic and Evolutionary Microbiology*, *58*(2), 375-382.

Scholz, H. C., Nöckler, K., Göllner, C., Bahn, P., Vergnaud, G., Tomaso, H., Al Dahouk, S., Kämpfer, P., Cloeckaert, A., Maquart, M., and Zygmunt, M. S. (2010). Brucella inopinata sp. nov., isolated from a breast implant infection. *International journal of systematic and evolutionary microbiology*, *60*(4), 801-808.

Seleem, M. N., Boyle, S. M., and Sriranganathan, N. (2010). Brucellosis: a re-emerging zoonosis. *Veterinary microbiology*, *140*(3), 392-398.

Sharifi, H., Mashayekhi, K., and Tavakoli, M. M. (2012). Risk facts of small ruminant Brucellosis: a cross-sectional study in southeast Iran. *Human and Veterinary Medicine*, *7*(1).

Sharifi, H., Tabatabaei, S., Rashidi, H., Kazeminia, S., Sabbagh, F., Khajooei, P., and Leontides, L. (2014). A cross-sectional study of the sero-prevalence and flock-level factors associated with ovine and caprine Brucellosis in southeastern Iran. *Iranian Journal of Veterinary Research*, *15*(4), 370-374.

Smits HL (2012). Control and prevention of brucellosis in small ruminants: time for action. *Veterinary Research*, *170*, 97–98.

Smits, H. L. (2013). Brucellosis in pastoral and confined livestock: prevention and vaccination. *Revue scientifique et technique (International Office of Epizootics)*, *32*(1), 219-228.

Sofian, M., Aghakhani, A., Velayati, A. A., Banifazl, M., Eslamifar, A., and Ramezani, A. (2008). Risk factors for human Brucellosis in Iran: a case–control study. *International Journal of Infectious Diseases*, *12*(2), 157-161.

Solorio-Rivera, J. L., Segura-Correa, J. C., and Sánchez-Gil, L. G. (2007). Sero-prevalence of and risk factors for Brucellosis of goats in herds of Michoacan, Mexico. *Preventive veterinary medicine*, *82*(3), 282-290.

Taleski, V., Zerva, L., Kantardjiev, T., Cvetnic, Z., Erski-Biljic, M., Nikolovski, B., … and Kirandziski, T. (2002). An overview of the epidemiology and epizootology of Brucellosis in selected countries of Central and Southeast Europe. *Veterinary microbiology*, *90*(1), 147-155.

Teshale, S., Muhie, Y., Dagne, A., and Kidanemariam, A. (2006). Sero-prevalence of small ruminant Brucellosis in selected Countys of Afar and Somali pastoral

areas of Eastern Ethiopia: the impact of husbandry practice. *Revue de médecine vétérinaire*, *157*(11), 557.

Thrusfield, M (2013). Veterinary epidemiology.New York: Elsevier.

Tsegay, A., Tuli, G., Kassa, T., and Kebede, N. (2015). Sero-prevalence and risk factors of Brucellosis in small ruminants slaughtered at Debre Ziet and Modjo export abattoirs, Ethiopia. *The Journal of Infection in Developing Countries*, *9*(04), 373-380.

Tsehay, H., Getachew, G., Morka, A., Tadesse, B., and Eyob, H. (2014). Sero-prevalence of Brucellosis in small ruminants in pastoral areas of Oromia and Somali regional states, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, *6*(11), 289-294.

Tsolis R.M. (2002). Comparative genome analysis of the alpha - proteobacteria: relationships between plant and animal pathogens and host specificity. *Proceedings of the National Academy of Sciences of the United States of America*, ,*99*(20), 12503-12505.

Verger, J. M., Grimont, F., Grimont, P. A., and Grayon, M. (1985). Brucella, a monospecific genus as shown by deoxyribonucleic acid hybridization. *International Journal of Systematic and Evolutionary Microbiology*, *35*(3), 292-295.

Verger, J. M., Grayon, M., Tibor, A., Wansard, V., Letesson, J. J., and Cloeckaert, A. (1998). Differentiation of Brucella melitensis, B. ovis and B. suis biovar 2 strains by use of membrane protein-or cytoplasmic protein-specific gene probes. *Research in microbiology*, *149*(7), 509-517.

Verger, J. M., Grayon, M., Cloeckaert, A., Lefèvre, M., Ageron, E., and Grimont, F. (2000). Classification of Brucella strains isolated from marine mammals using DNA–DNA hybridization and ribotyping. *Research in microbiology*, *151*(9), 797-799.

Wattam, A. R., Williams, K. P., Snyder, E. E., Almeida, N. F., Shukla, M., Dickerman, A. W., Crasta, O.R., Kenyon, R., Lu, J., Shallom, J.M., and Yoo, H. (2009). Analysis of ten Brucella genomes reveals evidence for horizontal gene transfer despite a preferred intracellular lifestyle. *Journal of bacteriology*, *191*(11), 3569-3579.

Xavier, M. N., Paixão, T. A., Poester, F. P., Lage, A. P., and Santos, R. L. (2009). Pathological, immunohistochemical and bacteriological study of tissues and milk of cows and fetuses experimentally infected with Brucella abortus. *Journal of comparative pathology*, *140*(2), 149-157.

Yesuf, M., Alemu, S., Temesgen, W., Mazengiac, H., and Negussie, H. (2012). Sero-prevalence of Ovine Brucellosis in South Wollo, North Eastern Ethiopia. *American-Eurasian Journal of Agricultural & Environmental Sciences*, *9*, 288-291.

Young E.J. (1995). An overview of human Brucellosis. *Clinical Infectious Diseases*, *21*, 283–289 (Quiz 290).

APPENDICES

Appendix 1: Questionnaire in English

The questionnaire was administered to the respondents with assistance of an interpreter who speaks the local Somali language. The interpreter was trained prior to the beginning of the investigation.

Questionnaire No: ……

Title: Sero-prevalence and Factors associated with Brucellosis in Goats and Sheep, Garissa County, 2013

Name of the farmer: .....

Name of interviewer: .....

Date of interview: .....

Time of interview: .....

Socio demographic data of respondent

Age in years:

.....

Gender:

.....

County:

.....

Division:

.....

Location:

.....

Sub-location:

.....

Village:

.....

GPS point:

Latitude.....Longitude.....Elevation.....

..

Highest level of formal attained education:

0=No formal education

1 = Primary

2 = Secondary

3 = College/graduate

5 = Other

Primary occupation:

1 = Employed full time on farm

2 =Employed part time on farm

3 =Self-employed off farm

4 =Employed off farm - agriculture

5 = Employed off farm

6= Other

Primary role:

1=Milking

2=Slaughtering

3 =Butchering

4= Cleaning barns

5= assisting in animal delivery

6= herding/

7=Feeding animals

8=Other

Laboratory information

What is the number of sheep and goats in the herd:

……Sheep……Goats

How many sheep and goats are sampled:

……Sheep…….Goats

How many are positive on RBPT:

……Sheep……Goats

From those positive on RBPT, how many are positive on CFT:

……Sheep……..Goats

Overall herd status:

Positive

Negative

Factors associated with Brucellosis in sheep and goats herds

1. Do you own other animals apart from sheep and goats? (If No skip to question 4)

   1=Yes

   2=No

2. If yes above, which ones and how many are they?

   Cattle……..

   Camels…….

   Donkey……

   Poultry…….

   Others (specify)…….

3. How do you raise your animals?

1=Raise them together

2=Raise them separately

4. Do you own all the sheep and goats in the herds? (If Yes, skip to question 6)

   1=Yes

   2=No

5. If No above, who else owns the animals in this herd?

   1=Neighbor

   2=Friend

   3=Family members

6. Do you have a calving pen for your herd?

   1=Yes

   2=No

7. Did your herd come into contact with other herds during grazing or watering in year?

   1=Yes

   2=No

8. Did your animals come into contact with wild animals in the past year?

   1=Yes

2=No

9. If yes, which wild animals?

   • Zebra

   • Buffalo

   • Antelopes

   • Waterbuck

   • Warthogs

   • Others (specify):

   ……

10. Have you ever found evidence of wildlife abortion in your farm or where you graze or water your animals?

    1=Yes

    2=No

11. Did you experience abortions or still births in your sheep and goats herd in the past year? (If No skip to question 12)

    1=Yes

    2=No

    If yes above, what was the number of animals aborting?

Sheep…….....

Goats…….....

12. Did you experience abortions in your other animals herd in the past year? (Skip if answer to question 1 was No and go to question 13)

    1=Yes

    2=No

    If yes, to above question which other animal(s) aborted and how many were they?

    Cattle ……..

    Camels …….

    Others……...

13. Did you introduce any new animal(s) into your sheep and goat herd in the past year through buying, dowry, compensations etc? (If No, skip to question 15)

    1=Yes

    2=No

    If yes above, How many were they?

    Sheep ……..

    Goats ……..

14. Was the animal(s) tested for diseases and quarantined before introduction?

1=Yes

2=No

15. Did you lend your male animals for breeding to other herds in past year?

    1=Yes

    2=No

16. Did you vaccinate your animals against infectious diseases in past year? (If No, skip to question 18)

    1=Yes

    2=No

17. If yes above, was Brucellosis one of the diseases you vaccinated against?

    1=Yes

    2=No

18. Did you get veterinary advice on management of your sheep and goats in past year?

    1=Yes

    2=No

Farmers Knowledge Attitude and Practices regarding Brucellosis

19. Have you heard of the disease called Brucellosis? (If No, questioning ends here)

    1=Yes

    2=No

20. If yes above, what do you think is the cause of this disease in animals?

    1= Food,

    2=Water,

    3=Wild animals,

    4=Sexual

    5=Witchcraft

    6= Hereditary

    7=Don't know

    8=Others (specify) …….

21. In your opinion, which animals are affected by Brucellosis? (Check all that are mentioned.)

    1=Goats

    2=Sheep

    3=Cattle

    4=Camels

5=Others (specify)

……

22. In your opinion, what are the common signs and symptoms of Brucellosis in sheep and goats? (Check all that are mentioned.)

    1=Abortions

    2=Retained placenta

    3=Swollen testes (orchitis)

    4=Infertility

    5=Metritis (Pus from Vulvular area)

    6=Arthritis/Hygroma (Swollen joint)

    7=Mastitis

    8=Others (specify):

    …….

23. Do you know whether animals can transmit Brucellosis to humans? (If No/don't know, skip to question 22)

    1=Yes

    2=No /don't know

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

1=Helping animals to deliver/abort by bear hands

2=Handling fetal tissues/aborted fetuses with bear hands

3=Drinking raw milk

4=Consuming products processed from raw milk

5=Slaughtering animals

6=Milking animals

7=Eating uncooked meat

8=Living with animals

9=Others (specify):

……

25. Have you ever been diagnosed with Brucellosis in the past?

1=Yes

2=No /don’t know

26. Have any of your family members been diagnosed with Brucellosis in the past?

1=Yes

2=No /don’t know

27. Have you seen any other person with Brucellosis in the past apart from your family member?

    1=Yes

    2=No/don't know

28. In your opinion, what are the signs/symptoms of Brucellosis in humans? (Check all that are mentioned.)

- Fever
- Headache
- Chills
- Fatigue
- Malaise
- Loss of appetite
- Joint pain
- Back pain
- Night sweating
- Diarrhea
- Nausea
- Vomiting
- Blurred vision
- Spontaneous abortion in women
- Painful scrotum in men

29. Where did you first learn about Brucellosis? (Check all that are mentioned.)

    1=Newspapers and magazines

    2=Radio

    3=TV

    4=Veterinary officials

    5=Brochures, posters and other printed materials

    6=Community Animal Health workers

    7=Family, friends, neighbors and colleagues

8=Religious leaders

9=Teachers

10=When I was diagnosed with the disease

11=Other (please explain):

……..

30. In your opinion, how serious a disease is Brucellosis in animals in your area? (Check one.)

    1=Very serious

    2=Somewhat serious

    3=Not very serious

31. In your opinion, how serious a disease is Brucellosis in humans in your area? (Check one.)

    1=Very serious

    2=somewhat serious

    3=Not very serious

32. Have you ever heard how you can prevent Brucellosis in animals?

    Yes

    No/Don’t know

33. How do you prevent animals from getting Brucellosis? (Please check all that are mentioned.)

    Vaccinations

    1=Isolation of sick or aborting animals from the rest of the herd

    2=Reporting to veterinary authority

    3=Don't know

    4=Other (please explain)

    …….....

34. Can Brucellosis be cured in humans? (If No, skip to question 36)

    1=Yes

    2=No/don't know

35. If yes above, how can someone with Brucellosis be cured? (Check all that are mentioned.)

    1=Herbal remedies

    2=Home rest without medicine

    3=Praying

    4=Specific drugs given by health facility

    5=Do not know

6=Others (specify):

……

36. What actions would you take if you were found out or suspected that your animals have Brucellosis? (Check all that are mentioned.)

1=Slaughter and consume the meat

2=Sell animal to a neighbor

3=Sell animal to a butcher

4=Sell animal in a market

5=Call a veterinarian

6=Do nothing

7=Others (specify)

…….

37. What action(s) would you take as regarding aborting animals in your herd? (Check all that are mentioned.)

1=Call a veterinarian

2=Self treat them

3=Separate them from the rest of the herd

4=Sell them

5=Slaughter them

6=Do nothing

7=Others

8=(specify)……
….

38. What would you do if you thought you had symptoms of Brucellosis? (Check all that apply.)

    1=Go to health facility

    2=Go to pharmacy

    3=Got to traditional healer

    4=Go to be prayed for

    5=Other (specify):
    ……

39. Have you assisted sheep and goats or any of your animals during parturition/abortion/removal of retained placenta in the past year? (If No/don't know skip to question 42)

    1=Yes

    2=No/don't know

40. Did you use any protective gloves or masks when assisting with the parturition or abortion of animals or whilst handling placentas and aborted fetuses?

    1=Yes

2=No/don’t know

41. How did you dispose of the aborted fetuses and placentas?

    1=Burning

    2=Dumping

    3=Burying

42. Did you participate in slaughtering/butchering of animals in past year?

    1=Yes

    2=No/don’t know

43. Did you participate in processing of raw milk products in past year?

    1=Yes

    2=No/don’t know

44. Did you consume raw milk or milk products made of raw milk in past year?

    1=Yes

    2=No/don’t know

45. Do you feel well informed about Brucellosis?

    1=Yes

    2=No

46. Do you wish you could get more information about Brucellosis?

    1=Yes

    2=No

47. What are the sources of information that you think can most effectively reach people like you with information on Brucellosis? (Please choose the three most effective sources.)

    1=Newspapers and magazines

    2=Radio

    3=TV

    4=Billboards

    5=Brochures, posters and other printed materials

    6=Health workers

    7=Family, friends, neighbors and colleagues

    8=Religious leaders

    8=Teachers

    9=Other (please explain):
    ……

Appendix 2: Consent form in English

Title of study:

Sero-prevalence and associated Factors for Brucellosis in Goats and Sheep, Garissa County

Introduction:

My name is Mark Odhiambo Obonyo. I am trying to learn more about Brucellosis. Brucellosis is a zoonotic disease that is of public health importance. It is transmitted from animals to humans when people get exposed to infected livestock and their products which may act as a source of infection.

Purpose of study:

Due to the great public health importance of this disease, I am requesting for your participation in this study whose main objective is to find out how many of your animals and others in this village and other villages in Garissa County are exposed to this serious disease, what are the factors associated with transmission or acquisition of this disease by your animals and also gauge your knowledge, attitude and practice. This is important for the relevant authorities to find ways of dealing with this disease in this area. You are being asked to join this study because your herd was picked by chance among other herds in this area.

Expectations of the study:

If you agree to participate in the study, I wish to test some of your animals to determine whether they could have been exposed to Brucellosis. If you agree to take part in the study, a trained laboratory technologist will withdraw 5-10mls of blood (that can fill a big spoon) from the jugular vein selected animals using a syringe and needle. The blood

specimen was transported to Garissa regional veterinary investigation laboratory where I was test for Brucellosis. I shall then ask you some questions which are written on a paper on animal husbandry and your knowledge, attitude and practices regarding Brucellosis. The test results shall be availed as soon as possible to County veterinary officer of Garissa who shall forward them to you and advice on any necessary control measures if need be.

Risks:

There are no envisaged risks from participating in this study. However minor bruising and bleeding may be noticed on the selected sheep and goats during withdrawal of blood sample.

Benefits:

The results of this study was communicated and disseminated to the people concerned for them to take action on the recommendations that was come out from the study results. This was include necessary control measures if need be.

Confidentiality:

Any information obtained from you was kept confidential and used solely for purposes of this research only. The results of this research may be published in scientific journals or presented at medical or veterinary meetings, but your identity will not be disclosed.

Compensation:

If you accept to take part in this study, there was no payment for participation.

Alternatives:

You have a choice to agree or not to agree to participate in this study. If you agree to participate in study you are allowed to withdraw from the study at any time if you so wish without any consequences whatsoever.

Approval of the study:

This study was approved by:

The Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee (KNH/UON-ERC)

P O BOX 20723-00202, Nairobi, Kenya

Email: uonknh_erc@uonbi.ac.ke

Tel: (254-020) 2726300 ext 44355 for UoN or 726300-9 for KNH

And

Board of Post graduate studies

Jomo Kenyatta University of Agriculture and Technology

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

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

Consent:

I have been fully informed about the study, the risks and benefits of it. I had the opportunity to ask questions which were satisfactorily answered. I therefore consent to voluntarily participate in the study.

Name of participant……

Signature/ thumb print of participant……..

Date……..

Name of researcher/research assistant……..

Signature…….. Date
…….

Appendix 3: Translated Questionnaire in Somali Language

LIFAAQA 1: SU'AALO

Cinwaanka : Sero-prevalence and iyo xaqiiqooyinka la xiriira cudurka Diis ee k u dhaca ariga iyo idaha , degmada Gaarisa ,2013

Magaca wareysi
qaadaha.....

Taariikhda
wareysiga.....

Waqtiga.....

GPS
point.....

Qayaas ahaan Inta u dhaxeysa GPS iyo goobta
xoolaha(KMs).....

Warbixinta Sheybaarka Meeqey dhan yihiin xoolaha ari iyo idaba
.....

Ari iyo ido meeqaa ayaa sample laga qaaday
?.....

Meeqaa RBPT positive noqotay
?.....

RBPT positive kuwa ah meeqaa sii ah CFT positive ?
.....

Muxuu yahay herd-level sero-positivity xoolaha
?.....

Meeqaa loo badalay herd-level sero-
positivity.....

XAQIIQOOYIN LA XIRII CUDURKA DIIS EE KU DHACA XOOLAHA SIDA ARIGA IYO IDAHA

1. Maleedahay xoolo kale markii laga reebo idaha iyo ariga?(hadii jaawantu tahay maya suaasha 4 ugudub)

   Haa……

   Maya……..

   Hadii jaawabta suaasha kore haa tahay , meeqaad leedahay waana xayawaankee/xoolohee?

   Lo'o…….

   Geel…….

   Dameero……

   Dooro……

   Kuwakale(fadlan sheeg)…….

2. Xoolaha side baad udhaqataa?

   Halmeel hal xero……..

Kala xero…….

3. Adi miyaa wada leh ariga iyo idaha meesha joogo?(hadii ee jaabtu tahay haa suaasha 6-aad ugub

   Haa……

   Maya……

4. Hadii jaawabtu tahay maya ,yaa kale oo kulaleh xoolaha?

……

……

……

5. Xoolaha maladaaqeen xoolo kale 6-dii bilood ee lasoo dhaafay?

   Haa…….

   Maya……

6. Xoolaha mala kulmeen xoolo kale markii ee biyaha cabaayeen?

   Haa,……

   Maya……

7. Xoolaha malakulmeen xayawaanka duurka 6-dii bilood ee soo dhaafay?

   Haa …….

   Maya……

8. Xoolahaga (idaha iyo ariga) dilan maku aragtay sanadkii lasoo dhaafay?

   (Hadii jaawabtu tahay maya ugudub saasha 11)

   Haa…….

   Maya……

9. Xoolaha aad ku aragtay dilan waa meeqaa?

   Idaha……

   Ariga……

10. Malakulantay dilan xoolohaada kale sanadkii lasoo dhaafay ?(hadii jawaabtu tahay maya ugudun suaasha 13)

    Haa……..

    Maya……..

11. Hadii jawaabtu suasha kore tahay haa waa xoolahee kuwa dilanka aad ku aragtay waana meeqaa?

    Lo'oda……
    ……

    Geela……
    ……

    Kuwakale……
    ……

12. Maku soo dartay xoolo cusub arigaada iyo idahaaga sanadkii lasoo dhaafay?(hadii jawaabtu tahay maya ugudub suaasha 17)

    Haa……..

    Maya…….

13. Meeqeey ahaayeen ?

    Idaha…….

    Ariga……

14. Xageed ka keentay xooloha?

    Suuqa……

    Dariska/jiiranka……

    Xoolodhaqato kale……..

    Meelo kale(fadlan sheeg)…….

15. Xooloha cusub mala baaray , gees malooxiray inta aan lagu darin xooloha?

    Haa,…….

    Maya…….

16. Maudhiibtay doorodaada(mida cagaha waaweyn) xoolo dhaqato kale sii eey utarmaan sanadkii lasoo dhaafay ?

    Haa……..

Maya……

17. Makatalaashay cudurada dilaaga ah xooloda sanadkii lasoo dhaafay?.(hadii jawaabtu tahay maya ugudub su'aasha 20)

Haa

Maya

18. Hadii jawaabtu suaahsa kore tahay haa ,Diis kamid ma'aheed kuwa aad katalaashay xooloha?

Haa

Maya

19. Dhaqtarka xooloha maku waaniyay sanadkii lasoo dhafay sidii aad udhaqi laheed ariga iyo idaha?

Haa…….

Maya…….

AQOONTA XOOLALEYDA ,SIDEY U ARKAAN ,QAABKEY ULA DHAQMAAN CUDURKA DIIS .SU'AALO GUUD IYO KUWA DADKA KU SAABSAN

20. Ma maqashay cudur la yiraahdo Diis ?(jawaabta hadey maya tahay ,su'aasha meeshaa ayey ku egtahay)

Haa……..

Maya……

21. hadeey jawaabta haa tahay maxaa xoolaha cudurkaan u keeno ?

    Germs……

    Kala dhaxlid…….

    Sixir…….

    Ma aqaano……..

    Kuwa kale (cadeey)……

22. Fikirkaada , cudurka Diis xayawaanadee ayuu ku dhacaa ?(dooro inta la sheegay)

    Ari……

    Ido…….

    Lo'……

    Geel ……

    Kuwa kale (
    sheeg)……..

23. Fikirkaada , calaamadahee lagu garanayaa Diis marku ariga iyo idaha yo (dooro kulli)

    Abortions/dilan…….

    Retained after birth/mandheer kuharto……..

    Orchitis/sheelo…….

Hygroma…….

Metritis…….

Arthritis/cudurka jilbaha…….

Mastitis/cudurka candhada xoolaha kagalo ……..

Kuwakale (fadlan sheeg):…….

24. Xoolaha ma u gudbin karaan dadka cudurka Diis?(maya/ma aqaano ubood su'aasha 34)

    Haa

    Maya /ma aqaano

25. Hadii jawaabta kore eey haa tahay , sidee? (dooro inta la sheegay)

    Ka dhalin xoolo gacmihiisoo banaan

    Gacantaada oon galoofis lahayn in aad xayawaan yar oo dhicis ku qabato.

    Cabid caano aan la karkarin

    Isticmaalid wax yaabo laga hagaajiyay caano aan karkarsaneyn

    Xoolo qalid

    Xoolo maalid

    Hilib aan la Karin cunidiisa

    Xoolo la noolaasho

Kuwa kale (sheeg): ……

26. Waligaa cudurka Diis makuugu dhacay ?

    Haa

    Maya / ma aqaano

27. Qof familkaada ka mid ah waligiisa ma qaaday cudurka Diis ka ?

    Haa

    Maya / ma aqaano

28. Qof aan familkaada ahayn waligaa Diis maku aragtay ?

    Haa

    Maya / ma aqaano .

29. fikirkaaga , calaamadahee lagu arkaa dadka markuu cudurka Diis ku dhaco ?

    Qandho

    Madax xanuun

    Daal

    qarqar

    xanuun aan la garaneynin/ caajis

    cuntadoo ka istaagta

Kalagoosyadoo xanuuno

Dhabar xanuun

Habeen dhidid

Shuban

Lalabo

Matag

Indhaha oo aragtida gaabiyo

Ilmo dilan

Raaya xanuun

30. Marki kugu horeysay Diis inteed ka baratay? (qor dhamaan inta la sheegay)

Newspapers and magazines

Radio

TV

Dhaqaatiirta xoolaha

Waraaqaha darbiyada lagu dhajiyo ama dadka loo qeybiyo

Shaqaalaha caafimaadka xoolaha ee bulshada dhaxdeeda

Familka , saaxiib , dariska ama qof wax aad wada barataan

Hogaamiyayaasha diimaha

Macaliminta

Markuu cudurka igu dhacay .

Kuwa kale (fadlan sharax) …….

31. Fikirkaada , sheeg qatarta uu cudurka Diis uu ku hayo xoolaha eeriyadaada? (dooro mal mid)

Halis

Halis aan sidaa ubadneyn

Halis ma jirto

32. Fikirkaaga , halis intee la eg ayuu cudurka Diis uu ku hayaa dadka eeriyadada? (dooro mid)

Halis

Dhaxdhaxaad /halis aan sidaa ubadneyn

Halis ma jirto

33. Sided uga hortagtaa si eeysan xoolaha u qaadin Diis ? (qor inta la sheegay)

Talaal

Ka takoorid inta kale

War galin dhaqaatiirta xoolaha

Ma aqaao

Kuwa kale (sheeg
)……

34. Diis dadka malaga daaweyn karaa? (jawaabta hadey maya tahay ubood S40)

Haa

Maya /ma aqaano

35. Hadey jawaabto haa tahay side loo daaweyn karaa ? (qor inta la sheegay)

    Daawo dhaqameed

    Nasiino ayadoon daawo la qaadan

    Salaad/duco

    Daawo eey kuu soo qoreen goobo caafimaad

    Ma aqaano

    Kuwa kale
    (Sheeg)……
    ……

    Sida loo arko iyo hab dhaqanka ku aadan Diis

36. Talaabo noocee ah ayaad qaadan laheyd hadi xoolahaaga laga heli lahaa ama looga shakiyo iney Diis qabaan ?(Qor kuli waxa la sheegay).

    Qal oo isticmaal hilibkooda

    Xoolaha ka gad dariska

    Kawaanka ka iibi xoolaha

    Suuqa ku iibi

    Uyeer dhaqtar xayawaan

    Wax ha suubin

Kuwa kale(xus)

……

37. Talaabo noocee ah ayaad qaadan lahayd oo ku aadan ilma dilanka xoolahaada?(qor inta la magacaabay)

Dhaqtar xoolo uyeerid

Adiga daaweyso

Xoolaha intooda kale gooni uga bixi

Iibi oo qal

Wax ha suuban

Kuwa kale (
xus)…….43: Maxaad suubin lahayd hadaad moodo in aad qabto calaamadaha Diis? (qor inta ku haboon)

Goob caafimaad aadid

Farmashye aadid

Daawo daqameed

Quraan aqris / duco saarid

Kuwa kale (xus)

……

38. Ma caawisay lax ama ri ama xoolahaada qeyb ka mid ah mar eey dhalayeen/ ilmo kasoo dilmeen/ mandheerta ka soo saartay sanadkii hore ?(hadey jawaabta maya tahay / ma aqaano u bood S47)

    Haa

    Maya / Ma aqaano

39. Ma isticmaashaa gloves ama masks marka aad mandheerta kasoo jiideysid ama aad ka dhalineysid ama ilmaha dilanka kasoo jiideysid ?

    Haa

    Maya / ma aqaano

40. Maxaad ku suubisaa dhiciska soo dilmo iyo mandheerta ?

    Gubid

    Qashinka ku darid

    Duugid

41. Maka qeyb gashay qalid / kawaan geyn xoolo lixdii bil ee lasoo dhaafay ?

    Haa

    Maya / ma aqaano

42. Maka qeyb qaadatay soo saarid caano aanan karkarsaneyn wax laga suubiyay lixdii bil ee laga soo gudbay ?

    Haa

Maya /ma aqaano

43. Caano aan la karkarin ma isticmaashay lixdii bil ee lasoo dhaafay ?

Haa

Maya /ma aqaano

44. Ma isticmaashay waxyaalo laga suubiyay caano aan la karkarin lixdii bil ee ugu danbeysay

Haa

Maya

Diis , aqoonisteeda iyo ilaha warbaxineed

45. Ma umaleynee in aad si fiican u taqaano Diis?

Haa

Maya

46. Ma rabtaa in warbixin dheeri aad ka hesho Diis ?

Haa

Maya

47. Maxey yihiin ilaha warbixin ee aad is leedahay warbixin fiican ayey dadka kaheli karaan oo ku saabsan Diis?(sheeg 3 qeyb ee ugu tayada roon).

Newspapers and magazines

Radio

TV

Dhaqaatiirta xoolaha

Waraaqaha darbiyada lagu dhajiyo ama dadka loo qeybiyo

Shaqaalaha caafimaadka

Familka , saaxiib , dariska ama qof wax aad wada barataan

Hogaamiyayaasha diimeed

Macaliminta

Kuwa kale (fadlan sharax) …….

Appendix 4: Translated consent form in Somali Language

LIFAAQA 3: Foomka ogolaanshaha

CINWAANKA BAARISTA

Sero –prevalence iyo xaqiiqooyinka la xiriira ee Diis ka ariga iyo idaha , Degmada Gaarisa

Hordhac:

Diis wuxuu ka midyahay cudurada loo yaqaano Zoonotic caafimaadka bulshadana muhiim u ah . xoolaha ayaa u gudbiya bini aadamka marka eey dadka isticmaalaan xoolo ama waxyaalo laga suubiyay xoolo qabay cudurka .

Ujeedada baaritaanka:

Waxaan kaa codsaneynaa kaqeybqaadahsada baaritaanka ee ujeedkiisu yahay in la qiimeeyo faafida iyo xaqiiqooyinka la xiriira cudurka Diis ee ku dhacay idaha iyo ariga ku nool Dagmada Gaarisa .

Waxa laga filanaayo baaristan :

Waxaan rajeyneynaa in xoolihiina qaar aan tijaabino in eey udhiban yihin Diis . hadaad nagala qeyb qaadato baaritaankan , waxaan dhiig yar ka qaadeynaa xoolaha aan dooranay kuwaas oon ka baareyno Diis , ka dib waxaan ku weydiineynaa suaalo dhowr ah oo ku saabsan xanaanada xoolaha iyo aqoonta aad uleedahay, sifooyinka iyo practiska ku saabsan Diis , baaritaanka natiijadiisa waxaa si dhaqsa leh loogu gudbinaa dhaqaatiirta xoolaha ee degmada gaarisa kuwaasoo idinka idinsoo gudbin doono idinna raacinayo waanooyin iyo waxyaalo kale oo loo baahan yahay .

Qatarta :

Ma jiraan Qatar la filanayo iney keento ka qeybqaadashada baaritaankam , si kastaba ha ahaatee dilaac yar ama diig yar inuu ka soo daato ayaa laga yaabaa ariga ama idaha marka dhiiga tijaabada laga qaadayo.

Faaiidooinka :

Natiijada kasoo baxeyso baaritaankaan waxaa loo gudbinayaa dadka uu quseeyo si eey u qaataan talaabooyinka ku haboon oo kasoo bixi doono baaritaanka.

Kalsooni:

Warbixin kasta oo dhankaaga ka timaato waa la xifdinayaa waxaa loo isticmaalaaya dano baaris oo kaliya , natiijada kasoo baxdo baaritaankaan waxaa lagu daabacayaa scientific journals ama waxaa loo gudbin dhaqtar ama kulan dhaqaatiir xooleed laakin qofka aad tahay waa la qarinayaa.

Fursada aad heysato

Shaqadada barista ah waqtigaad doonto ayaad ka bixi kartaa wax dhibaata ahna ma jirayaan.

Ogolaanshaha baaritaanka :

Baaritaankaan waxaa ogolaaday gudiga:

The Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee (KNH/UON-ERC)

P O BOX 20723-00202, Nairobi, Kenya

Email: uonknh_erc@uonbi.ac.ke

Tel: (254-020) 2726300 ext 44355 for UoN or 726300-9 for KNH

Iyo

Board of Post graduate studies

Jomo Kenyatta University of Agriculture and Technology

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

Ogolaansho:

Si wacan baa leygu sheegay baaritaanka ,qatarta iyo faaiidooyinka , waxaan heystay fursad aan suaalo ku weydiin karay kuwaa oo jawaabo aan ku qancay lahaa ,sidaa darted waxaan ogolahay inaan si voluntarily ah uga qeybqaato baaritaankan

Magaca ka qeyb qaataha

……

Saxiixa / suulka ka qeybqaataha

……

Taariikhda

……..

Magaca baaraha /caawiye bare

……..

Saxiixa

……...

Taariikhda

…….

Signature……..

Date……

Appendix 5: Manuscript on Sero-prevalence and herd level factors associated with Brucellosis in sheep and goats in Garissa County, 2013.

Manuscript Published in BMC Veterinary

Title: Estimated Brucellosis sero-prevalence at individual animal and flock level in Sheep and Goats and factors associated with flock level sero-positivity in Garissa County, Kenya-2013

Running Title: Brucellosis Sero-prevalence and associated factors, Kenya

Author: Mark Obonyo[1, 2, 3§] and Gideon Kikuvi[2]

Author Affiliations:

[1]Field Epidemiology and Laboratory Training Program, Ministry of Health, Kenya

[2]Jomo Kenyatta University of Agriculture and Technology, School of Public Health

[3]Ministry of Agriculture, Livestock and Fisheries, Directorate of Veterinary Services, Kenya

[§]Corresponding author

Email addresses:

MO: mobonyo@feltp.or.ke

GK: kikuvi@yahoo.com

Abstract

Background: Brucellosis, a zoonosis of major public health importance, is endemic in livestock in Kenya. A cross-sectional epidemiological study was carried out to estimate the sero-prevalence of brucellosis and identify factors associated with flock level sero-positivity in small ruminants in Garissa County of North Eastern Kenya.

Methods: A total of 2,400 sera from 120 flocks were collected from sheep and goats which were randomly selected using a multi-stage sampling technique and data on potential flock level factors were collected from livestock owner or their representative ≥15 years using a pre-tested structured questionnaire. The sera were analyzed using Rose Bengal Plate Test (RBT) and sero-positive reactors confirmed by Complement Fixation Test (CFT) using serial interpretation. We considered a sample to be positive when both tests results were positive and a flock was considered positive when a single animal within the herd tested positive on both tests. Multivariate logistic regression was used to investigate for independent factors associated with flock brucellosis sero-positivity in small ruminants.

Results: The overall sero-prevalence of brucellosis at individual animal-level was 20.0% [(394/2400); (95% CI: 18.2% to 22.0%)]; in goats 24.3% [(290/1471); (95% CI: 21.8% to 27.1%)] and sheep 12.5% [(104/979) (95% CI: 10.2% to 15.2)]. Overall flock-level sero-prevalence was 65.8% [(62/120) (95% CI: 54.3% to 77.2%)]. Seeking veterinary services [aOR=0.30 (95% CI: 0.12 to 0.76], introduction of new animals into the flock [aOR=8.0 (95% CI: 3.09 to 20.70] and experiencing abortions in the flock [aOR=3.43 (95% CI: 1.33 to 8.88] were independently associated with brucellosis flock sero-positivity in small ruminants.

Conclusions: The study highlights considerable high sero-prevalence of brucellosis and factors that contributes for its transmission in small ruminants in Garissa County. This

poses potential public health threat associated with zoonotic transmission. We recommend a one health approach for effective control of brucellosis in this region.

Key words: Brucellosis, Risk factors, Sero-positivity, Kenya, Garissa, Small Ruminants

Background

Brucellosis, more so that caused by *B. melitensis* remains one of the most common zoonotic diseases worldwide accounting for an estimated 500,000 human cases reported annually [1]. The true incidence of human brucellosis worldwide is estimated to vary from <0.03 to >160 per 100,000 population [1, 2]. The bacteria is classified by the US Centers for Disease Control and Prevention as a category (B) pathogen which can be developed into a bio-terrorist agent of mass human destruction with a potential of aerosol transmission [3].

Brucellosis infection in small ruminants causes heavy economic losses due to production and reproduction losses resulting from mass abortions, neonatal losses, reduced fertility, and decreased milk production, high cost of veterinary care and costs associated with replacement animals. In addition, the disease also hinders free animal movement due to imposition of quarantines to affected farms and it is a great impediment for international trade of animals and their products [4]. The disease in humans is associated with high direct and indirect medical and non-medical expenses arising from high cost of treatment of primary disease and management of sequelae as well as productivity losses [5].

Brucellosis is endemic is several parts of Kenya and there is increasing evidence that the incidence and prevalence is increasing both in humans and animals more so in pastoral regions [6-8]. Since domestic animals are the source of infection to humans, increasing incidence and prevalence in humans is perhaps a reflection of a similar trend in domestic animals. Livestock plays a crucial role in the livelihood of the majority of

residents of Garissa who are predominantly nomadic pastoralists keeping mainly sheep, goats, cattle and camels. It is estimated that livestock sector provides employment to almost 95% of the residents of Garissa County and it is the main source of milk and meat and plays an important role in many socio-cultural traditions in this set up. Sheep and goats are considered as cash banks which can easily be liquidated to meet urgent family needs. Between 2005 and 2007, the sector generated an estimated 1.8 billion Kenya shillings from direct sales in domestic and overseas markets [9]. However this dependence on livestock makes these people vulnerable to zoonotic diseases. Some of the socio cultural practices such as consumption of raw milk has been shown to make residents of Garissa at greater risk of infection with brucellosis [6].

Although there are no published studies that incriminate Brucella species as cause of abortion in goats and sheep in Garissa County, brucellosis has been suspected on basis of clinical grounds. Unfortunately, reliable data on brucellosis in Kenya is scarce and data on sero-prevalence and risk factors associated with small ruminant brucellosis in Garissa County is unknown. Understanding of sero-prevalence of brucellosis, geographical distribution and risk factors for its transmission in mall ruminants is vital for designing effective control strategy [10]. This study aimed at determining the sero-prevalence of brucellosis in sheep and goats at individual animal and flock level and to identify factors associated with brucellosis at flock level with an aim of providing evidence based information to inform prevention and control strategies in both domestic animals and humans in this region.

Materials and Methods

Study site and study population

We conducted the study in Garissa County which is classified as arid and semi-arid and has an altitude range of between 70m and 400m above sea level. Human population is estimated at 623,060 and combined sheep and goat population at 1,322,457

according to the 2009 Kenya housing and population census. The region has two rain seasons (April to May and October to December) with annual mean precipitation of 225mm. The area is characterized by hot and dry weather throughout the year and daily temperatures are typically above 30 °C (86 °F), while at night, they can fall to 20 °C (68 °F). Indigenous people of Somali origin account for the majority of the population in Garissa County [9, 11].

Study Design

We conducted a cross-sectional study between October and November 2013 in flocks that we randomly selected from 36 sub-locations in Garissa Township and Balambala sub-counties of Garissa County. We calculated sample size using Thrushfield formula for simple random sampling [12] as shown below:

$$n = \left(\frac{1.96}{d}\right)^2 \times p(1-p) \qquad (1)$$

We assumed an expected flock prevalence of 16% in goats [7], a 10% level of precision of the estimate, a confidence interval of 95% and a design effect of two because of the multi-stage sampling approach used to give a minimum sample size of 103 flocks. In addition, we applied a 10% contingency to give rise to 114 flocks.

We obtained a list of households from all the sub-locations in Garissa and Balambala sub-counties with estimated numbers of sheep and goats owned by each household. This was achieved with assistance from the local national government administrators, community elders, community animal health workers (CAHWs) and the Garissa County veterinary and livestock production office. We considered the sheep and goats flocks as the primary sampling unit (PSU) and used the household lists as proxy for the number of flocks in each sub-location. Due to logistic issues, we randomly selected a third of the sub-locations (12 sub-locations) for inclusion into the study. We randomly selected the number flocks to sample in each sub-location proportionate to the

number of flocks in each sub-location. Once we determined the number of flocks per sub-location to be included in the study, we employed systematic random sampling (SRS) technique to select flocks within each sub-location for sampling using the livestock owners' list as sampling frame. We attempted a simple random sampling to select individual animals within the flock for blood sample collection. We sampled up to a maximum of 20 animals per flock and we selected sheep and goats for sampling proportionate to their numbers within the flock. Since sheep and goats are raised together as one flock in this set up, the same sample size applied to both. We included flocks whose owners (or representative) were willing to participate in the study; allowed blood samples to be collected from the flock and were ≥15 years of age. We replaced flocks whose owners/representative not meeting the inclusion criteria with another flock among the remaining flocks in the list from the same sub-location to achieve the required sample size for a particular sub-location.

Sample collection

The owner and field assistants individually restrained sheep and goats selected for sample collection and we aseptically collected10–15 ml of blood into plastic vacutainers®. We allowed the vacutainers® to stand for approximately 15 to 30 minutes in a rack in a slanting manner at ambient temperature to separate serum from clot. We harvested the sera using disposable plastic Pasteur pipettes and dispensed into Eppendorf tubes and stored in a cool-box containing ice cubes in the field. We transported the Eppendorf tubes to the laboratory where the specimens were stored in a freezer at -20°C until used for serological testing.

Sample analysis

We screened for presence of antibodies in collected sera specimens using Rose Bengal Plate test (RBT) and complement fixation test (CFT). The sensitivity and specificity of RBT were 89% and 97%, respectively and that of CFT were 88% and

100%. The CFT and RBT test antigens *(Brucella arbortus* strain 99*)*, control sera and other reagents were obtained from Atlas medical, William James House, Cowley Rd. Cambridge Cb4, 4WX and sensitized sheep red blood cells (SRBC) were obtained from the central veterinary laboratory (national veterinary reference laboratory in Kenya). The sera specimens were tested serially first using RBT then CFT for those that tested positive on RBT. An animal was considered positive if the serum specimen tested positive on both RBT and CFT whereas a flock was considered positive if at least a single serum specimen from an animal within the flock tested positive on both RBT and CFT. For RBT, Rose Bengal test antigen was prepared from killed standard strain of B. abortus strain 99 and stained with Rose Bengal dye, in an acidic buffer pH 3.65. Serum samples and the antigen were left at room temperature for an hour before the test commenced. We shake the bottle containing the antigen so that the suspension was homogeneous. We thereafter added 30µl of the sample after swirling for a minute onto a white tile and same volume of antigen alongside the antigen spots using a micro-pippete. We then thoroughly mixed the sera and antigen using a wooden splint, using one wooden splint for each test, until a circular zone of approximately 2 cm was formed. We thereafter rocked the white tile both clockwise and anti-clockwise for four minutes (timing was done using a laboratory buzzer). We then observed for any agglutination (due to antigen and antibody complex formation) in a well-lit place to avoid false positive reading due to formation of fibrin. We used a magnifying glass to examine those tests that were suspected to have micro-agglutination. We considered a positive test results as any visible agglutination and negative results as absence of any visible agglutination. We tested a control serum that provides minimal agglutination every day before the actual testing began to verify the sensitivity of the test conditions. For CFT, we diluted the test serum and appropriate working standards with equal volume of veronal buffered saline in small tubes and incubated at 58°C for 50 minutes to inactivate the native complement. We thereafter dispensed 25µl of diluted test serum on a round bottom 96 well micro-titre on the first and second rows of the well. We then added 25µl of the veronal buffered saline to all wells except those on the first row. We applied

serial doubling dilution by transferring 25µl of serum from third row onwards and discarded 25µl of the mixture in last row. We carried out the serial dilution four times. We then dispensed 25µl of antigen to each well except in the first row followed by 25µl of complement to each well. We set up control wells having diluent only; control wells having complement and diluent; and control wells having diluent, complement and antigen each at 75µl volume in each control wells. We tested control serum that results into a minimum positive reaction for each set of tests to ascertain the sensitivity of test conditions. We then incubated the plates at 37°C for 30 minutes and thereafter added 25µl of sensitized sheep red blood cells (SRBC) to each well. We then re-incubated the plates at 37°C for 30 minutes. Thereafter we centrifuged the plates for 100rpm for 10 minutes to allow the SRBC that did not undergo hemolysis to settle. We compared degree of hemolysis with standard s corresponding to 0, 25, 50, 75 and 100% hemolysis. We also checked for the absence of complementary activity for serum in the first row. We considered sera samples having SRBCs sedimentation at a dilution ≥ 1:5 to be positive for Brucellosis. We conducted both RBT and CFT tests as described and recommended by OIE [13].

We determined the true prevalence (TP) at individual animal level and flock level using a formula by Rogan and Gladen [14] and combined test sensitivity and specificity of the two tests when interpreted serially which we calculated as 78% and 100% respectively [15].

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1} \qquad (2)$$

Data collection

We administered a pre-tested structured questionnaire to the flock owner or their representative immediately after bleeding the animals to collect information on potential flock level factors associated with small ruminant brucellosis. We interviewed the

participants in their local language by assistance from a trained research assistant. We translated the questionnaire from English to Somali language (local language) and back translated to English using professional independent translators to ensure consistency, clarity and socio-cultural acceptability by the community. We pre-tested the questionnaire on five flocks and made adjustments, additions and modifications to the questions. The flock level factors we collected included: flock size; contact with wildlife and wildlife abortus; introduction of new animal into the flock in the past one year; keeping other livestock apart from sheep and goats; history of abortion in livestock kept in past one year; seeking veterinary services and lending male animals to other farmers for breeding.

Data management

We entered cleaned and analyzed data from questionnaires on flock-level factors and laboratory results using Epi Info 7 (CDC, Atlanta, GA, USA) and Ms. Excel 2007 (Microsoft, Seattle, WA, USA). We calculated descriptive statistics for relevant variables. For proportions, we estimated the 95% confidence interval (CI) using the exact binomial test. To evaluate the association between the flock level factors and Brucella sero-positivity, we carried out univariable analysis where we calculated prevalence odds ratios (PORs), 95% confidence intervals (95% CI) and p-values. Factors with p-value of ≤ 0.05 were considered statistically significant. We used unconditional multiple logistic regression employing a stepwise forward selection approach to identify independent factors associated with flock level Brucella sero-positivity. We calculated adjusted odds ratios (aOR), 95% CI and p-values. For selection of independent variables for inclusion into the initial multiple logistic regression model, the entry criterion was p-value ≤ 0.20. We developed the model by dropping the least significant independent variable until all the remaining predictor variables were significant (p-value ≤ 0.05). All biologically and statistically plausible two-way interactions between variables remaining in the final model was tested and retained if significant.

Results

Individual animal level sero-prevalence

We sampled a total of 2,400 animals composed of 979 (41%) sheep and 1471 (59%) goats from 120 flocks having a total of 12,945 animals. The median flock size was 112 animals (range: 58 to 140 animals). Of the 2,400 sera samples examined, 437 (18.2%); (95% CI: 16.7% to 19.8%) were positive for Brucella antibodies on RBT. Of the 437 samples positive on RBT, 138/979 (14.1%); (95% CI: 12.1% to 16.4%) were from sheep and 299/1471 (20.3%); (95% CI: 18.4% to 22.5%) were from goats. Of the samples testing positive on RBT, 394/2400 (16.4%); (95% CI: 15.0% to 18.0%) tested positive on CFT of which 104/979 (10.6%); (95% CI: 8.8% to 12.7%) were from sheep and 290/1471 (19.7%); 95% CI: 17.8% to 21.8%) from goats.

Adjusted overall true sero-prevalence of Brucellosis at individual animal level in sheep and goats was 20.0% (95% CI: 18.2% to 22.0%); and in goats it was 24.3% (95% CI: 21.8% to 27.1%) and sheep 12.5% (95% CI: 10.2% to 15.2%). At individual animal level, highest apparent sero-prevalence of 37.8% [(68/180); (95% CI: 30.92% to 45.03%)] was recorded in Abdisimit; 25.4% [(61/240); (95% CI: 20.21% to 31.21%)] in Ohio and 22.5% [(63/280); (95% CI: 17.9% to 27.67%)] in Kuno. The lowest apparent sero-prevalence of 8.5% [(17/200); (95% CI: 5.2% to 13%)] were recorded in Sikley and 2% [(6/300); (95% CI: 0.82% to 4.11%)] in Balambala **(Table 1).**

Flock level sero-prevalence

At the flock level, the overall apparent flock sero-prevalence was 51.7% (62/120); 95% CI: 42.8% to 60.4%) and the overall true flock sero-prevalence of brucellosis was 65.8% (95% CI: 54.3% to 77.2%). The apparent flock level sero-prevalence varied by sub-location with the highest 85.7% [(6/7); (95% CI: 47.0% to

99.3%)] recorded in Galbet and the lowest 13.3% [(2/15); (95% CI: 2.3% to 37.5%)] recorded in Balambala **(Table 2).**

Flock level factors associated with Brucellosis sero-positivity

In determining both the risk factors and protective factors associated with brucellosis flock sero-positivity, we examined 10 factors at the univariate analysis of which five variables (introduction of new animal in the flock, experiencing abortion in sheep and goats flock, contact with other flocks, lending male animals to other flocks for breeding purposes and seeking veterinary services) were significantly associated with brucellosis flock sero-positivity with p values ≤ 0.05. The following factors were independently associated with brucellosis sero-positivity in sheep and goats at flock level: introduction of new animals into the flock [adjusted odd ratio (aOR) = 8.0; 95% CI: 3.1 to 20.7], experiencing abortion in sheep and goats flock (aOR = 3.4; 95% CI: 1.3 to 8.9) and seeking of veterinary services (aOR = 0.3; 95% CI: 0.1 to 0.8) **(Table 3).**

Discussion

We report the serological analysis of exposure to *Brucella spps* in sheep and goats and determination of factors associated with sero-positivity at the flock level from a population based sample of sheep and goats flocks in Garissa County. The study estimated the true sero-prevalence of *Brucella spps* in sheep and goats both at the individual animal and flock level incorporating the sensitivity and specificity of the diagnostic tests used and the uncertainties in these tests. The large uncertainty in the estimates of sero-prevalence especially at the flock level could be attributed to the small sample size of flocks included in this study from sub-locations sampled. However, the result from this study does confirm that there is very high level of probability that the sheep and goats flocks in Garissa have been exposed to *Brucella spp.* The study also highlights the need for adjusting for the sensitivity and specificity of serological tests

used in serological surveys for reliable interpretation of results. This also provides unbiased estimates of sero-prevalence estimates.

Brucellosis sero-prevalence in the sheep and goats population in Garissa County appears to be high at 20.0% individual animal level and 65.8% at flock-level. Several studies have found very variable small ruminant brucellosis sero-prevalence at individual and flock-level across various African countries. Estimates include animal-level sero-prevalence of between 0.4% and 9.7%, and 15% flock-level sero-prevalence in Ethiopia [16-29]; 16% individual animal and 20% flock-level sero-prevalence in Cameroon [30]; between 0% and 8.2% individual animal and 0% and 56.3% flock sero-prevalence in Eritrea [31]; between 0.4% and 3.6% individual animal and 17.8% flock-level sero-prevalence in Niger [32]; between 0.44% and 12.1% individual animal level in Egypt [33, 34]; individual animal level of 3.3% in sheep and 4.5% in goats in Nigeria [35]; between 0.9% and 22% individual animal level in Sudan [36-38] and in Uganda, 4% individual animal and 13% flock-level sero-prevalence in goats were estimated [39]. In Kenya, studies done in Kiambu, Kajiado and Marsabit Counties estimated animal-level sero-prevalence ranging from 1.3% to 16.1% and flock level sero-prevalence ranging from 5.6% to 68% [7]. Another study done in Kenya in Baringo County estimated an individual animal sero-prevalence of 13.04% in goats and 8.23% in sheep [8]. These variations in estimates of sero-prevalence of brucellosis in small ruminants may be largely attributed to the varied animal husbandry systems, various agro-ecological zones in which the studies were carried out, the sampling methodology employed and diagnostic tests used. These factors have been shown to contribute to variation in the obtained sero-prevalence of brucellosis infection in livestock among different researchers [40-43]. However the high sero-prevalence of brucellosis in sheep and goats in Garissa County portends greatest probability of zoonotic transmission of brucellosis to humans.

The introduction of new animals into the sheep and goats flock from unscreened flocks was significantly associated with flock seropositivity in this study. This was in

agreement with several other studies which noted that introduction of animals from non-free brucellosis flocks or from flocks of which their brucellosis status was unknown was significantly associated with brucellosis in sheep and goat flocks and in cattle [39, 44-48]. Other studies suggest that the introduction of infected animals can lead to an increase in the individual level prevalence due to the fact that the longer the animals stay in the flock and they are in contact with rest of the flock, the higher the risk of spread of brucellosis would be [49, 50]. Practices that involve movement of animals between flocks have also been found likely to be risky and potentiate transmission of brucellosis between flocks. Evidence suggests that failure of most brucellosis control strategies could be attributed to the lack of control in the movement of animals and measures to avoid introduction of infected animals by maintaining a completely closed flock or by carefully screening purchased animals before introducing them into the flock, a practice that is very rare in pastoral communities, is perhaps one of the effective control strategies for small ruminants brucellosis [39, 44, 51].

Presence of female animals who had aborted in the flock was found to be significantly associated with flock sero-positivity. Abortion in livestock represents the major complaint attributed to Brucella infections [39, 52-55]. Females infected with *Brucella spp*s are known to shed highly concentrated volumes of Brucella organisms in their milk, placental membranes and aborted fetuses and continue shedding the organisms for several months resulting into environmental contamination [4]. As a result, there is enhanced high risk of transmission of Brucella organisms between animals of the same flock and other flocks during free mixing in grazing and watering places. There is also enhanced zoonotic transmission to humans. During this study, we also assessed the livestock owners knowledge attitude and practices towards brucellosis as reported elsewhere [56] where most of those interviewed reported assisting animals during abortions and birthing processes and handling fetal materials without any protective clothing putting them at risk of coming down with the disease should such materials be contaminated with Brucella organisms. Similarly the owners reported

consuming unpasteurized milk and milk products and just dumping aborted fetuses and fetal membranes, practices which clearly shows their ignorance of the epidemiology of the disease.

Lending male animals to other flocks for breeding purposes and seeking veterinary services in the past year were also factors that were significantly associated with brucellosis flock sero-positivity with the former being a risk factor and the latter a protective factor. Lending of male animals for breeding has been identified in other studies to be a risk factor associated with Brucella sero-positivity in animals [57, 58]. Although venereal route is not considered an important route for Brucella transmission in small ruminants under natural conditions, practices that involve movement of animals between flocks are considered risky due to potential of mechanical transmission [4, 59].

We utilized a cross-sectional study design which was not ideal for investigation of flock level factors associated with brucellosis in sheep and goats in this region. The study design could not permit the investigators to determine when the animals within the flocks classified as positive were first exposed or when the Brucella organisms were first introduced into the flock. This may have led to misclassification of the flock level factors associated with seropositivity. However, since the study aimed to identify associations and not cause-effect relationships; we deemed that the design was sufficient in identifying the flock level factors associated with Brucellosis in this region despite the small numbers of flocks included in the study. We only focused on small ruminants' brucellosis despite the fact that other livestock species such as such as cattle and camels which are also reared in this community were never studied and they are deemed to be susceptible to brucellosis. We were also not able to identify the various species and *biovars* of *Brucella spps* circulating in in sheep and goats as evaluate the socio-economic impact of the disease in limiting livestock production.

Conclusion and Recommendation

Despite the limitations highlighted above, the present study confirms a considerably high seroprevalence of Brucellosis both at individual animal and flock level in sheep and goats. The introduction of new non-quarantined animals with unknown brucellosis status into the flock, occurrence of abortions within the flock (which is could be a pointer to brucellosis infection) and lending of male animals for breeding purposes were significant risk factors associated with the spread of the disease within and between flocks whereas seeking veterinary care as found to be a protective factor. Necessary measures should therefore be taken to prevent the transmission of brucellosis to the human population. Future studies in this region should holistically focus on all animal species including humans to provide a more comprehensive picture of the disease epidemiology and socio-economic implications.

The pastoral lifestyle of the community in the study area makes brucellosis control very challenging not only because of the number and complexity of risk factors involved, but also because the risk factors are tightly linked and often inherent to the livestock production practices. The above factors when combined with the close interaction between the people and their livestock coupled with the socio-cultural lifestyle and poor knowledge attitude and practices [56] evidenced in this study, poses a serious public health concern and has been known to enhance transmission of brucellosis to the human population. These reasons and make control of brucellosis both in livestock and humans challenging.

There are no officially coordinated program for control of brucellosis in Kenya and at large, greater horn of Africa where Kenya belongs. However, the African Union Inter-African Bureau on Animal Resources (AU-IBAR) developed a strategy dubbed "Standard Methods and Procedures (SMPs) for control of Brucellosis in the Greater Horn of Africa" which outlines minimum standards, methods and procedures on various subject areas such as surveillance, laboratory procedures and disease control that must be met for harmonized regional control of the disease [60]. If fully adopted, these SMPs will go a long way towards control of livestock brucellosis in the greater horn of Africa.

However for the full potential of the SMPs to be realized, there is need to evaluate attitudes of communities involved and their roles need to be clearly defined in the various control strategies. For effective control of this disease in this region, a "One Health" approach is recommended.

Abbreviations

RBT: Rose Bengal Plate Test; CFT: Complement Fixation Test; POR: Prevalence odds ratio; aOR: Adjusted odds ratio; AU-IBAR: African Union Inter-African Bureau on Animal Resources; SMP: Standard Methods and Procedures

Declarations

Acknowledgements

We would like to extend our thanks to Kenya Field Epidemiology and Laboratory Program (K-FELTP) for financial support to carry out the study. The authors would also wish to acknowledge all the staff of the Department of Veterinary Services and Livestock production of Garissa County and staff at Regional Veterinary Investigation Laboratory in Garissa for their cooperation and untiring efforts towards the success of this study. We would also acknowledge the study participants and the community leaders for support provided during the study.

Funding

This study was fully funded by Kenya Field Epidemiology and Laboratory Training Program who provided full scholarship for the first author to pursue a master's degree in applied epidemiology from Jomo Kenyatta University of Agriculture and Technology. The funding body of this study did not participate in the design or conclusion of the study.

Availability of data and materials

The datasets used and/or analyzed during the current study are available as Additional file 1.

Authors' Contribution

Both the authors' conceived and designed the study. MO collected, cleaned the data, performed the experiments and analyzed the data. MO drafted the manuscript. GK helped with the interpretation of the results. All authors' read, critically reviewed and approved the final manuscript.

Ethics approval and consent to participate

We sought and obtained approvals for the study protocol from Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and we obtained ethical clearance from Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee (KNH-UoN ERC) under approval number P248/05/2013. We explained the study objectives to all potential study participants in their local language (Somali) and obtained informed oral consent from all those who agreed to participate in the study. We obtained ethical approval for verbal consent from KNH-UoN ERC and documented consent from each consenting study participant on the questionnaire for data collection. We independently interviewed each study participant and measures were taken to ensure that collected data was properly stored, secured and confidentiality maintained.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no personal or financial competing interests that may bias publication of this manuscript.

Author Information:

Dr. Mark Obonyo-Field Epidemiology and Laboratory Training Program, Ministry of Health, Kenya and Ministry of Agriculture, Livestock and Fisheries, Directorate of Veterinary Services, Kenya

Prof. Gideon Kikuvi-Jomo Kenyatta University of Agriculture and Technology, School of Public Health

References

1. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV: **The new global map of human brucellosis**. *The Lancet infectious diseases* 2006, **6**(2):91-99.

2. Taleski V, Zerva L, Kantardjiev T, Cvetnic Z, Erski-Biljic M, Nikolovski B, Bosnjakovski J, Katalinic-Jankovic V, Panteliadou A, Stojkoski S: **An overview of the epidemiology and epizootology of brucellosis in selected countries of Central and Southeast Europe**. *Veterinary microbiology* 2002, **90**(1):147-155.

3. Seleem MN, Boyle SM, Sriranganathan N: **Brucellosis: a re-emerging zoonosis**. *Veterinary microbiology* 2010, **140**(3):392-398.

4. Radostits OM, Gay CC, Hinchcliff KW, Constable PD: **Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats**: Elsevier Health Sciences; 2006.

5. Al-Majali AM: **Seroepidemiology of caprine brucellosis in Jordan**. *Small Ruminant Research* 2005, **58**(1):13-18.

6. Kiambi SG: **Prevalence and factors associated with brucellosis among febrile patients attending Ijara District Hospital, Kenya**. 2014.

7. Osoro EM, Munyua P, Omulo S, Ogola E, Ade F, Mbatha P, Mbabu M, Nganga Z, Kairu S, Maritim M: **Strong Association Between Human and Animal Brucella Seropositivity in a Linked Study in Kenya, 2012–2013**. *The American journal of tropical medicine and hygiene* 2015:15-0113.

8. Kosgei P, Bebora L, Waiboci L, Kitala P, Kiambi S: **Estimating prevalence of brucellosis in livestock and assessment of knowledge, attitudes and practices of respective communities in baringo county, Kenya**.

9. **Garissa County Profile** [www.garissa.go.ke]

10. Smits H: **Brucellosis in pastoral and confi ned livestock: prevention and vaccination**. *REVUE SCIENTIFIQUE ET TECHNIQUE-OFFICE INTERNATIONAL DES EPIZOOTIES* 2014, **32**(1).

11. **Kenya Population and Housing Census** [www.knbs.go.ke]

12. Thrusfield M: **Veterinary epidemiology**: Elsevier; 2013.

13. OIE: **Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Accessed from: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.04_BRUCELLOSIS.pdf. Date of access: 04/02/2018**. In.; 2016.

14. Rogan WJ, Gladen B: **Estimating prevalence from the results of a screening test**. *American journal of epidemiology* 1978, **107**(1):71-76.

15. **Epitools** [www.epitools.ausvet.com]

16. Ashagrie T, Deneke Y, Tolosa T: **Seroprevalence of caprine brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia**. *African Journal of Microbiology Research* 2011, **5**(13):1682-1685.

17. Bekele M, Mohammed H, Tefera M, Tolosa T: **Small ruminant brucellosis and community perception in Jijiga District, Somali Regional State, Eastern Ethiopia**. *Tropical Animal Health and Production* 2011, **43**(4):893-898.

18. Ferede Y, Mengesha D, Mekonen G: **Study on the seroprevalence of small ruminant brucellosis in and around Bahir Dar, North West Ethiopia**. *Ethiopian Veterinary Journal* 2011, **15**(2).

19. Ibrahim N, Belihu K, Lobago F, Bekana M: **Sero-prevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia**. *Tropical Animal Health and Production* 2010, **42**(1):35-40.

20. Megersa B, Biffa D, Abunna F, Regassa A, Godfroid J, Skjerve E: **Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia**. *Trop Anim Health Prod* 2011, **43**:651-656.

21. Negash E, Shimelis S, Beyene D: **Seroprevalence of small ruminant brucellosis and its public health awareness in selected sites of Dire Dawa region, Eastern Ethiopia**. *J Vet Med Animal Hlth* 2012, **4**:61-66.

22. Teshale S, Muhie Y, Dagne A, Kidanemariam A: **Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia: the impact of husbandry practice**. *Revue de médecine vétérinaire* 2006, **157**(11):557.

23. Mohammed S, Tuli G, Nigatu S, Alemaw G: **Sero-Prevalence of Brucellosis in Goats Purchased for Slaughter in Selected Export Abattoirs, Ethiopia**. 2015.

24. Tsehay H, Getachew G, Morka A, Tadesse B, Eyob H: **Seroprevalence of brucellosis in small ruminants in pastoral areas of Oromia and Somali regional states, Ethiopia**. *Journal of Veterinary Medicine and Animal Health* 2014, **6**(11):289-294.

25. Tsegay A, Tuli G, Kassa T, Kebede N: **Seroprevalence and risk factors of Brucellosis in small ruminants slaughtered at Debre Ziet and Modjo export abattoirs, Ethiopia**. *The Journal of Infection in Developing Countries* 2015, **9**(04):373-380.

26. Dabassa G, Tefera M, Addis M: **Small ruminant brucellosis: Serological survey in Yabello district, Ethiopia**. *Asian Journal of Animal Science* 2013, **7**:14-21.

27. Negash E, Shimelis S, Beyene D: **Seroprevalence of small ruminant brucellosis and its public health awareness in selected sites of Dire Dawa region, Eastern Ethiopia**. *Journal of Veterinary Medicine and Animal Health* 2012, **4**(4):61-66.

28. Yesuf M, Alemu S, Temesgen W, Mazengiac H, Negussie H: **Seroprevalence of Ovine Brucellosis in South Wollo, North Eastern Ethiopia**. *Am Eurasian J Agric Environ Sci* 2010, **9**:288-291.

29. Deddefo A, Sisay T, Tuli G: **Seroprevalence and risk factors of small ruminant brucellosis in selected districts of Arsi and East Shoa zones, Oromia region, Ethiopia**. *African Journal of Microbiology Research* 2015, **9**(19):1338-1344.

30. Scolamacchia F, Handel IG, Fèvre EM, Morgan KL, Tanya VN, Bronsvoort BMdC: **Serological patterns of brucellosis, leptospirosis and Q fever in Bos indicus cattle in Cameroon**. *PLoS One* 2010, **5**(1):e8623.

31. Omer M, Skjerve E, Holstad G, Woldehiwet Z, Macmillan A: **Prevalence of antibodies to Brucella spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems**. *Epidemiology and Infection* 2000, **125**(02):447-453.

32. Boukary AR, Saegerman C, Abatih E, Fretin D, Bada RA, De Deken R, Harouna HA, Yenikoye A, Thys E: **Seroprevalence and potential risk factors for Brucella spp. Infection in traditional cattle, sheep and goats reared in urban, periurban and rural areas of niger**. *PloS one* 2013, **8**(12):e83175.

33. Hegazy Y, Molina-Flores B, Shafik H, Ridler A, Guitian F: **Ruminant brucellosis in upper Egypt (2005–2008)**. *Preventive veterinary medicine* 2011, **101**(3):173-181.

34. Hegazy YM, Moawad A, Osman S, Ridler A, Guitian J: **Ruminant brucellosis in the Kafr El Sheikh Governorate of the Nile Delta, Egypt: prevalence of a neglected zoonosis**. *PLoS Negl Trop Dis* 2011, **5**(1):e944.

35. Brisibe F, Nawathe D, Bot C: **Sheep and goat brucellosis in Borno and Yobe states of arid northeastern Nigeria**. *Small Ruminant Research* 1996, **20**(1):83-88.

36. Gumaa M, Osman H, Omer M, El Sanousi E, Godfroid J, Ahmed A: **Seroprevalence of brucellosis in sheep and isolation of Brucella abortus biovar 6 in Kassala State, Eastern Sudan**. *Rev Sci tech Off Int Epiz* 2014, **33**:957-965.

37. Abdallah AA, Elfadil AAM, Elsanosi EM, Shuaib YA: **Seroprevalence and Risk Factors of Brucellosis in Sheep in North Kordofan State**. 2015.

38. Omer MM, Abdelaziz AA, Abusalab SM, Ahmed AM: **Survey of brucellosis among sheep, goats, camels and cattle in Kassala area, Eastern Sudan**. *Journal of Animal and Veterinary Advances* 2007.

39. Kabagambe E, Elzer P, Geaghan J, Opuda-Asibo J, Scholl D, Miller J: **Risk factors for Brucella seropositivity in goat herds in eastern and western Uganda**. *Preventive Veterinary Medicine* 2001, **52**(2):91-108.

40. Mangen M, Otte J, Pfeiffer D, Chilonda P: **Bovine brucellosis in sub-Saharan Africa: estimation of sero-prevalence and impact on meat and milk offtake potential**. *Food and Agriculture Organisation of the United nations, Rome* 2002.

41. McDermott JJ, Arimi S: **Brucellosis in sub-Saharan Africa: epidemiology, control and impact**. *Veterinary microbiology* 2002, **90**(1):111-134.

42. Nielsen K: **Diagnosis of brucellosis by serology**. *Veterinary microbiology* 2002, **90**(1):447-459.

43. Díaz-Aparicio E, Marin C, Alonso-Urmeneta B, Aragón V, Pérez-Ortiz S, Pardo M, Blasco J, Diaz R, Moriyon I: **Evaluation of serological tests for diagnosis of Brucella melitensis infection of goats**. *Journal of clinical microbiology* 1994, **32**(5):1159-1165.

44. Refai M: **Incidence and control of brucellosis in the Near East region**. *Veterinary microbiology* 2002, **90**(1):81-110.

45. Coelho AM, Coelho AC, Góis J, de Lurdes Pinto M, Rodrigues J: **Multifactorial correspondence analysis of risk factors for sheep and goat brucellosis seroprevalence**. *Small Ruminant Research* 2008, **78**:181-185.

46. Bamaiyi PH, Hassan L, Khairani-Bejo S, ZainalAbidin M, Ramlan M, Krishnan N, Adzhar A, Abdullah N, Hamidah NHM, Norsuhanna MM: **Case–control study on risk factors associated with Brucella Melitensis in goat farms in Peninsular Malaysia**. *Tropical animal health and production* 2014, **46**(5):739-745.

47. Sharifi H, Mashayekhi K, Tavakoli MM: **Risk facts of small ruminant brucellosis: a cross-sectional study in Southeast Iran 2012**. *Human & Veterinary Medicine* 2015, **7**(1).

48. Sharifi H, Tabatabaei S, Rashidi H, Kazeminia S, Sabbagh F, Khajooei P, Karamouzian M, Nekouei O, Adeli Sardooei M, Leontides L: **A cross-sectional study of the seroprevalence and flock-level factors associated with ovine and caprine brucellosis in southeastern Iran**. *Iranian Journal of Veterinary Research* 2014, **15**(4):370-374.

49. Corbel MJ: **Brucellosis in humans and animals**: World Health Organization; 2006.

50. Rahman AA, Saegerman C, Berkvens D, Fretin D, Gani MO, Ershaduzzaman M, Ahmed MU, Emmanuel A: **Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal Test and Slow Agglutination Test for the diagnosis of brucellosis in sheep and goats in Bangladesh**. *Preventive Veterinary Medicine* 2013, **110**(2):242-252.

51. MacMillan A, Nielsen K, DUNCAN J: **Animal brucellosis**. *Animal brucellosis* 1990.

52. Muma J, Godfroid J, Samui K, Skjerve E: **The role ofBrucellainfection in abortions among traditional cattle reared in proximity to wildlife on the Kafue flats of Zambia**. *Revue Scientifique et Technique-Office International des Epizooties* 2014, **26**(3).

53. Muma J, Samui K, Oloya J, Munyeme M, Skjerve E: **Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia**. *Preventive veterinary medicine* 2007.

54. Musa M, Eisa M, El Sanousi E, Wahab MA, Perrett L: **Brucellosis in camels (Camelus dromedarius) in Darfur, Western Sudan**. *Journal of comparative pathology* 2008, **138**(2):151-155.

55. Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M, Zinsstag J: **Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad**. *Preventive veterinary medicine* 2003, **61**(4):279-293.

56. Obonyo M, Gufu WB: **Knowledge, Attitude and Practices towards Brucellosis among Pastoral Community in Kenya, 2013**. *International Journal of Innovative Research and Development* 2015, **4**(10).

57. Al-Majali AM, Majok AA, Amarin NM, Al-Rawashdeh OF: **Prevalence of, and risk factors for, brucellosis in Awassi sheep in Southern Jordan**. *Small Ruminant Research* 2007, **73**(1):300-303.

58. Reviriego F, Moreno M, Domınguez L: **Risk factors for brucellosis seroprevalence of sheep and goat flocks in Spain**. *Preventive Veterinary Medicine* 2000, **44**(3):167-173.

59. Benkirane A: **Ovine and caprine brucellosis: World distribution and control/eradication strategies in West Asia/North Africa region**. *Small ruminant research* 2006, **62**(1):19-25.

60. **Standard Methods and Procedures (SMPs) for Control of Brucellosis in the Greater Horn of Africa. Nairobi.** [www.au-ibar.org/component/jdownloads/finish/76-tmt/2116-standard-methods-and-procedures-smps-for-control-of-brucellosis-in-the-greater-horn-of-africa]

Appendix 6: Manuscript on Brucellosis KAP survey

Published in International Journal of Innovative Research and Development, September, 2015 Vol 4 Issue 10

Title: Knowledge, Attitude and Practices towards Brucellosis among pastoral community in Kenya, 2013

Running Title: Pastoralism, Brucellosis and Knowledge, Attitude and Practices

Author: Mark Obonyo[1, 2]☐ and Waqo Boru Gufu[1, 3], Gideon Kikuvi[2]

Affiliations:

[1]Field Epidemiology and Laboratory Training Program

[2]Jomo Kenyatta University of Agriculture and Technology

[3]Ministry of Health, Kenya

☐**Corresponding author:** markobonyo@yahoo.com

Abstract

Background: Brucellosis is a global zoonotic disease and a major public and animal health problem in many parts of the world, particularly in pastoral set up where livestock is a major source of livelihood and food. Effective prevention and control of Brucellosis depends on knowledge, attitude and practices of the community. This study aimed to assess the knowledge, attitudes and practices related to Brucellosis among pastoralists in Garissa.

Methods: The study was based on a cross-sectional study design, using a multistage sampling technique and a structured questionnaire was administered using a face-to-face interview to farmers aged 15 years and above.

Results: A total of 120 pastoralists were interviewed of which 90 (75%) were male; median age was 16 years (Range: 15 – 70 years); 102 (85%) were aged below 35 years and 95 (79%) had heard of Brucellosis. Among those aware of Brucellosis, 17 (18%) mentioned bacteria/germ as cause and 44 (46%) were informed through community health workers. Abortion was mentioned by 56 (59%) of respondents as main clinical sign of Brucellosis in animals. Sixty-seven (71%) knew Brucellosis as zoonotic disease of which 55 (82%) mentioned drinking of raw milk as main route of transmission. Fever was mentioned by 71 (75%) as main clinical symptom. Regarding attitudes and perceptions, 13 (14%) knew that Brucellosis could be prevented in animals; 33 (35%) knew that it could be treated in humans; only eight (8%) would visit a health facility if they suspected Brucellosis and 44 (46%) would do nothing if they had aborting animal in their herd. Regarding practices, 91 (96%) consumed raw milk in past year; 72 (76%) assisted an animal during birthing process of which 61 (75%) disposed fetal materials by dumping; and 34 (36%) participated in slaughtering an animal.

Conclusions: The study indicates that Brucellosis remains a major public health problem among the pastoralists in this area. Though the community has fair knowledge on Brucellosis, attitudes, perceptions and practices are poor. The study highlights the

importance of increased provision of information about knowledge attitude and practices regarding Brucellosis in this area as one of the major strategies in prevention and control of Brucellosis.

Key words: Knowledge; Attitude; Practice; Pastoralism; Brucellosis

Introduction

Brucellosis remains amongst the most normally disregarded zoonotic diseases worldwide[1, 2]. The true incidence of Brucellosis in human and animals worldwide is obscure and the occurrence is expanding in low and middle income nations like Kenya [3, 4]. The bacterial pathogen is considered by US Centers for Disease Control and Prevention (CDC) as a category (B) pathogen that has potential for improvement as a bio-terrorism weapon with a capability of airborne transmission [5].

In animals, Brucellosis is thought to be a group or herd issue spread inside of the herd fundamentally by ingestion of contaminated materials. Venereal infection can likewise happen, primarily with *B. Suis*. Congenital (in utero) or perinatal infection might likewise happen often resulting into latent infection. Spread between herds normally happens by introduction of asymptomatic chronically sick animals. Initial infection in female animals results in abortion and in long term, delayed or permanent infertility. The disease is considered chronic and infected animals continue to shed Brucella organisms following abortions, after subsequent parturitions and also in milk and colostrum [6].

Human transmission occurs through breaks in the skin following direct contact with contaminated animal tissues like blood, urine, vaginal discharges, aborted fetuses or placentas. Foodborne transmission occurs more often from consumption of raw milk and raw milk products like cheese and yoghurt. However once in a while eating raw meat from infected animals may result into infection. Brucellosis is considered an occupational hazard and airborne transmission has been documented among personnel working in laboratories and among abattoir workers. Accidental inoculation with live vaccine (such as B. abortus Strain 19 and B. melitensis Rev.1) can likewise happen. Cases of venereal and congenital infections are also known to occur in humans [7, 8]. Incubation period following infection with Brucellosis in human varies from few days to several years. This is followed by clinical signs and symptoms mostly characterized by intermittent or undulant fever, headaches, weakness, profuse sweating, chills, depression and weight loss [9].

Brucellosis represents various difficulties in designing effective prevention and control programs. This is primarily due to the chronic or asymptomatic nature of the disease in both animals and humans, varied incubation period and often lack of laboratory confirmation[10, 11]. In pastoral communities where Brucellosis is of most prominent significance due to close interaction between the pastoralists and their animals, population of animals in such set up are usually ill defined or unknown [10]. In Kenya, Brucellosis is endemic in several parts of the country and evidence exists of increasing incidence and prevalence in both human and animals especially in pastoral areas in Kenya [12, 13]. Given that infected animals are the source of human infection, the increasing prevalence of human Brucellosis probably reflects a similar trend in domestic animals. Due to nomadic pastoral lifestyle of the study community there is frequent mixing of animals on common grazing grounds or at water sources ensures maintenance of infection within and between herds. The eating habits and lifestyle of pastoralist also enhance transmission of Brucellosis in humans thus making control of Brucellosis both in livestock and humans challenging [14, 15]. Effective Brucellosis control programs depends on understanding of prevalence, geographical distribution, risk factors for transmission and knowledge, attitude and practices of livestock owners [14]. Limited information is available on knowledge, attitude and practices of livestock farmers in this set up. This study aimed at assessing the knowledge, attitude and practices of sheep and goat farmers and owners in Garissa County in order to provide evidence based information geared towards prevention and control of Brucellosis both in animals and humans.

Methods

Study area and population

The study was conducted in the pastoral County of Garissa which is low lying with altitudes ranging between 70m and 400m above sea level. The county is generally semi-arid and receives annual rainfall of between 150mm and 300mm. The communities in this region derive their livelihoods by selling livestock and livestock products and by

product, and recently they have started growing food crops especially along river Tana which traverses the County. The rainfall in this area is unreliable and main sources of water for both livestock and humans are mainly permanent water points like bore holes, dams and seasonal shallow wells. The temperatures in the county are high ranging from 20^{o}C to 38^{o}C [16]. Human population is estimated as 623,060 and combined sheep and goat population is 1,322,457 animals [17] .

Sampling Procedure and sample size determination

Between October and November 2013, a cross sectional survey was conducted in randomly selected households located in 36 sub-locations of Garissa County to assess the knowledge and perception of the communities about Brucellosis. With the help of the Local national government administrators, local elders, community animal health workers and veterinary office, the research obtained a list of households from all the sub-locations with estimated numbers of sheep and goats owned by each household. The primary sampling unit was the sheep and goats herds and the household lists acted as proxy for the number of herds in each sub-location. Due to logistic issues, a third of the sub-locations (12 sub-locations) from the 36 listed were selected using simple random sampling technique. Number of herds to sample in each sub-location was randomly selected proportionate to the number of herds in each sub-location. Once the number of herds per sub-location was determined, a systematic random sampling technique was used to select herds within each sub-location for sampling using the generated livestock owners list as a sampling frame. Study eligibility was based on willingness to be interviewed and being more than or equal to 15 years. Any livestock owner or representative not meeting any of the criteria was replaced with another farmer or representative from the sub-location list until the desired sample size in each sub-location was achieved. Sample size was estimated at 114 herds using a 16% herd prevalence of Brucellosis in goats in Kajiado County [13], a 10% level of precision of the estimate, a design effect of 2 due to multi-stage sampling technique employed. Thrushfield formula for simple random sampling was employed [18].

Data management

Information on knowledge about Brucellosis which included awareness of Brucellosis, sources of information on awareness, causes , awareness of Brucellosis as a zoonotic disease, mode of transmission in both animals and humans, signs and symptoms in animals and human and prevention and control measures in animals and treatment in humans. Information collected on attitude and perceptions included attitude and perception on seriousness of Brucellosis in human and animals, attitude towards prevention of Brucellosis in animals and treatment in humans, attitude towards aborting animals and attitude and perceptions when someone suspects to have Brucellosis. Information on practices included consumption of raw milk or milk products made from raw milk, participation in slaughtering or butchering an animal, assisting an animal during birth or removal of retained placenta or abortion and method of dumping of foetal materials after birth or abortion. Information on the socio-demographic characteristics of the participants was also included in the questionnaire. The questionnaire was translated from the original English version into the local language (Somali) and back translated to English by independent persons to ensure consistency, clarity and socio-cultural acceptability in the community. During pre-testing, additional information was gathered and some of the questions were modified. The participants were interviewed in their local language by the principal investigator and trained research assistant. We entered and cleaned the data using Microsoft Excel 2010 (Microsoft, Seattle, WA, USA), and analyzed using Epi Info version 7 (CDC, Atlanta, GA, USA) and Microsoft Excel 2010. We calculated proportions for categorical variables and means and medians for continuous variables.

Ethical issues

The study protocol approval was sought and obtained from Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and ethical clearance was sought and obtained from Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee (KNH-UoN ERC). The study objective was

explained to participants in their local language (Somali) and informed oral or written consent was obtained from each study participant who agreed to participate. Each participant was interviewed independently and measures were taken to assure collected data were properly stored, secured and confidentiality maintained.

Results

Socio-demographic characteristics of participants

A total of 120 participants were interviewed to assess their knowledge, attitude and practices towards Brucellosis. The median age of the study participants was 16 years (Range: 15 – 70 years), with 102 (85%) aged below 35 years. There were 90 (75%) males; and 92 (77%) had no formal education. In regard to primary role of the study participants in management of the herd, 58 (48%) were herd owner, 38 (32%) were herders and 24 (20%) were involved mostly in milking animals in the herd. Eighty-three (69%) of the participants were married and 37 (31%) were single **(Table 1).**

Awareness and cause of Brucellosis in animals and humans

Among the study participants, 95 (79%) had heard of Brucellosis, 17 (18%) mentioned germs/bacteria as the cause of Brucellosis, 38 (40%) did not know the cause, 14 (15%) mentioned food, and 13 (14%) mentioned water and wild animals. Among those who had heard of Brucellosis, 44 (46%) was through community health workers, 19 (20%) from a family member, 19 (20%) religious leaders, eight (8%) from veterinary staff and five (5%) from local FM stations **(Table 2).**

Knowledge of respondents'about the animals affected and signs/symptoms of Brucellosis in animals

In regard to animal species affected by Brucellosis, 62 (65%) mentioned goats, 47 (49%) sheep, 45 (47%) cattle and 32 (34%) camels. Fifty-six (59%) of respondents mentioned abortion as most common sign, 21 (22%) mentioned retaned placenta, 20 (21%) swollen

joints or hygroma and 11 (12%) mentioned mastitis or swollen udder and teats **(Table 3)**.

Knowledge of respondents on modes of transmission of Brucellosis to humans

Concerning Brucellosis being zoonotic disease, 67 (71%) of the respondents knew of this. Among these, 55 (82%) mentioned drinking raw milk as most common mode of transmission of Brucellosis from animals to humans, followed by eating milk products from raw milk mentioned by 27 (41%) respondents. The least mode of transmission of Brucellosis from animals to humans was slaughtering animals mentioned by 17 (26%) respondents **(Figure 2).**

Knowledge of respondents on signs/symptoms of Brucellosis in humans

Forty-six (48%) of the respondents knew a family member who had been diagnosed with Brucellosis in the past, 43 (45%) knew somebody who is not a family member who had been diagnosed with Brucellosis and 38 (40%) respondents had themselves been diagnosed with Brucellosis in the past. Concerning signs and symptoms in humans, 71 (75%) of respondents mentioned fever, 56 (59%) joint pains, 48 (51%) muscle pains, 45 (47%) loss of appetite and 38 (40%) chills **(Table 4)**.

Respondents' attitude/perception towards Brucellosis

A total of 63 (67%) of the respondents thought that Brucellosis is a serious disease in animals whereas 61 (64%) thought that it is a serious disease in human. Only 13 (14%) respondents thought that Brucellosis can be prevented in animals. Among these, six (46%) mentioned vaccination, four (39%) contacting veterinary office and three (31%) isolation of sick/aborting animals. In regard to treatment of Brucellosis in humans, 33 (35%) thought that Brucellosis in human can be treated/cured. Out of these, 14 (42%) mentioned visiting a health facility, eight (24%) seeking divine intervention (prayers), six (18%) consuming herbal medicine and five (15%) would purchase medicine from a local chemist. When confronted with an aborting animal in the herd, 44 (46%) will do

nothing, 16 (17%) would treat the animal with antibiotics, 11 (12%) will sell the aborting animal, 10 (11%) will consult veterinary office for advise, eight (8%) will isolate the animal from the herd and five (5%) will slaughter the animal **(Table 5)**.

Respondents' self-reported practices' towards Brucellosis

Regarding practices towards Brucellosis, a total of 91 (96%) of the respondents consumed raw milk in the past year, 72 (76%) assisted an animal during birthing process or abortion or removal of retained placenta, 46 (48%) introduced new animals into sheep and goat herd, 34 (36%) participated in slaughtering/butchering an animal, 30 (34%) lend their male animals to other farmers for breeding and 13 (14%) consumed milk products processed from raw milk and. Among those who assisted an animal during the birthing process, 61 (75%) disposed of fetal material by dumping and none used any protective clothing **(Table 6)**.

Sources on more information on Brucellosis

A total of 92 (97%) of the respondents believed that they were not sufficiently informed about Brucellosis and required more information on Brucellosis. The most favored mode of receiving information on Brucellosis was through the local FM radio stations mentioned by 36 (39%) of respondents, 23 (25%) favored religious leaders, 18 (20%) local community meetings (barazas) and 15 (16%) community health workers/community animal health workers **(Figure 3).**

Discussion

The results of this community-based cross-sectional study showed that Brucellosis is known by the general community in the present study area, since more than three quarters of the study respondents had heard of Brucellosis. This is similar to findings of previous studies done in Uganda among pastoral communities living along lake Mburo; in Egypt among cattle and Buffalo farmers in a village in Nile Delta region and among small ruminant farmers in the peri-urban areas of Dushanbe Tajikistan in which 99.3%,

83.2% and 57% of the respondents' had heard of Brucellosis. However, the awareness of Brucellosis among study participants in Uganda and Egypt were higher compared to our study but that in Tajikistan was lower [19-21]. In contrast to this finding, a study done among small-scale dairy farmers in an urban and peri-urban area of Tajikistan and another one done among urban and peri-urban dairy and non-dairy farming households in Kenya found that most respondents had not heard of Brucellosis. In the Kenyan study, 30% of dairy respondents and 22% of non-dairy respondents knew of the existence of Brucellosis whereas in Tajikistan 85% of the respondents had never heard of Brucellosis [22, 23]. Perhaps an explanation as to why the pastoral communities are more aware of Brucellosis compared to farmers in urban or peri-urban areas could be due to their close proximity and interaction with animals resulting into in-built indigenous knowledge over years which is subsequently passed down from one generation to the next. Despite a higher proportion of the study participants had heard about Brucellosis, majority had little or no knowledge about the cause of the disease. Less than a fifth of the participants correctly mentioned germ/bacteria as cause of Brucellosis. Poor knowledge regarding etiology of Brucellosis could negatively impact on respondents' preventive and control methods of Brucellosis in both humans and animals due to misconception on the cause.

The main sources of information on Brucellosis in this study area was community health workers (CHWs) followed by family members. Contrary to this finding, the study in Uganda and the two studies in Tajikistan found main source of information to be from friends/relatives [19, 21, 22]. Few participants in the current study mentioned mass media (radio/TV) as a source of information about Brucellosis, which was similar to the studies in Uganda and Tajikistan. This findings implies the powerful role the community health volunteers play in terms of relaying important health messages to nomadic pastoralists in this area who in most circumstances have challenges in accessing basic health care services. Deliberate moves should therefore be undertaken to incorporate the two in all aspects of health care education for the pastoralists.

Based on results of this study, the respondents' had basic knowledge about the animal species affected and signs/symptoms of Brucellosis in animals. In this regard, about two

thirds mentioned goats, close to a half sheep and cattle, and majority were not aware that camels could be affected. This findings contrast with the findings of studies in Tajikistan [22] where 82% of respondents knew that cattle, sheep and goats could be affected and the study in Egypt [20] in which 98.1% mentioned cattle, 86% sheep and 85% goats. However, our study was fairly in agreement with another study in Tajikistan [21] in which two thirds mentioned that all animals could be affected. With regards to clinical signs of Brucellosis in animals, more than half of the respondents mentioned abortion as the major clinical sign. This finding was in agreement with findings of a study done among pastoralists in Kaduna state in Nigeria and the study in Egypt in which 94.4% and 59.5% of respondents mentioned abortion as the major clinical sign [20, 24]. However our finding was different from that done in Tajikistan where only 11% of respondents' mentioned abortion as a clinical signs of Brucellosis in animals [21]. Knowledge of the animal species affected and signs/symptoms of Brucellosis in animals are crucial because it positively impacts on farmers' practices towards prevention and control measures of Brucellosis in both animals and humans.

More than two third of our study participants knew that Brucellosis is a zoonotic disease, findings which were similar to those in previous studies conducted in Tajikistan, Egypt, Nigeria and Uganda [20-22, 25]. However, our findings contrasted those of studies done in Ghana and Nigeria which found very low awareness of zoonotic nature of Brucellosis [24, 26]. In our study, among those who were aware of the zoonotic nature of Brucellosis, consumption of raw milk and raw milk products and handling of aborted fetuses were the top most modes of transmission of Brucellosis from animals to humans. The respondents' response regarding consumption of milk as a mode of transmission was comparable to findings in Egypt and Uganda [19, 20]. However in the current study, the respondents' had low awareness on other modes of transmission such as handling of aborted fetuses and fetal membranes, consumption of raw or undercooked meat, assisting animals during parturition and slaughtering animals; most of which have been identified in many studies as major risk factors for transmission of Brucellosis from animals to humans [27, 28]. Such low knowledge on mode of transmission of

Brucellosis from animals to humans has been documented elsewhere [21, 24, 26]. Good knowledge of mode of transmission of Brucellosis from animals to humans has been shown to have a protective effect towards human infection as shown in a hospital based matched case control study done in Kyrgyzstan [29].

In the current study, the majority of the study participants identified fever, joint pains and muscle pains in that order as the major signs and symptoms of Brucellosis. This was consistent with the findings of a previous study in Kyrgyzstan where fever and joint pain (locally known as "Tajik") were mentioned as main signs and symptoms of Brucellosis in humans [21] as well as a study in Nigeria where all respondents knew signs and symptoms of Brucellosis in humans [25]. However, the finding of the current study is different from the results of previous studies conducted in other parts of Nigeria and in Ghana [24, 26] where almost all participants were not aware of signs and symptoms of Brucellosis in humans. The respondents' basic knowledge about the signs and symptoms of Brucellosis in humans could have significant impact if the community knowledge is enhanced thus reducing diagnosis and treatment delay which in the long run will prevent sequelae and prolonged human suffering.

The present study showed that a considerable proportion of the study respondents perceived that Brucellosis was a serious disease in both animals and humans. However, despite this high perception of risk, most respondents' had unfavorable attitude towards prevention of Brucellosis in animals and treatment of Brucellosis in suspected humans. Regarding respondents' opinion on actions most would take when confronted with an aborting animal in their herd, majority would do nothing about it whereas others would attempt treating the animal with antibiotics or sell the animal. Very few mentioned isolation of the animal or seeking veterinary services. Failure to isolate suspected animals has been cited as one of the major risk factors for transmission of Brucellosis within and between herds as susceptible animals can become infected through contact with infected animals aborted tissues or consumption of pasture or water contaminated with aborted materials [30]. Frequent migration of pastoralists with their animals increases the chances of different herds coming into contact with other potentially

infected herds thus spreading diseases [31, 32]. This is more important when considering the high levels of infectiousness of Brucella species making practices such as sharing grazing land and drinking water points by pastoral communities a major transmission pathway of Brucellosis between different herds [33-35]

The study participants indicated that the communities in the present study area are engaged in risky practices that could expose them to infection with Brucellosis. Nearly all respondents consumed raw milk, about three quarter assisted animals during abortions or parturition and handled aborted materials/fetal membranes and a third participated in slaughtering or butchering an animal. Of those who assisted aborting animals, three quarter dumped the aborted materials and none used any protective clothing. Such risky practices have been shown to be important risk factors for Brucellosis transmission to human [12, 27, 28, 36, 37]. Female animals infected with *Brucella* spp. excrete high concentrations of the organism in their milk, placental membranes and aborted fetuses [6, 30]. Goats have also been shown to have prolonged secretion of Brucella organisms in milk compared to sheep [38]. Furthermore, Brucella species have been shown to survive in aborted fetuses, manure and water for periods of up to 150 to 240 days [39]. Therefore, there is a high risk of transmission of the pathogen between animals and from animals to humans through direct contact with contaminated materials such as fetal membranes, aborted fetuses, manure and other animal products. Introduction of new animals into the herd without quarantine and borrowing or lending breeding males to other farmers or even taking a female to be served at a neighbor's farm have been identified as major risk factors for transmission of Brucellosis within and between herds as shown in studies in several places [40-46].

Limitations of the study

Although the present study provides important information on the knowledge, attitude and perception of the pastoralists in Garissa County, it has limitations. The major limitation of the study was the small sample size which could affect the power of the study and external validity of the findings making it impossible to generalize findings

even to the whole of Garissa County except the villages which were included in the study. Self-reporting on practices by the respondents was also subject to recall bias and the face-to-face-interview situation, while enabling full response rates on all variables as well as participation of livestock keepers most of whom were illiterate, might have additionally enhanced this type of bias in assessing attitudes and behaviors.

Conclusion

The results of this study revealed that pastoralists in the study area had low level of knowledge about the causative agent but some moderate knowledge on the main symptoms of Brucellosis in animals and human. In addition, the study showed moderate level of overall knowledge, unfavorable attitude and poor practices towards Brucellosis. At present, there is no officially coordinated program for control of Brucellosis in Kenya. Understanding of the knowledge, perceptions and practices have been defined as important pillars regarding the feasibility and the acceptability of potential measures that might be instituted. Enhanced public health education on the cause, symptoms and mode of transmission of Brucellosis would be important towards the prevention and control of Brucellosis in the present study area. This can be achieved by targeted messages in local FM radios and integrating the community health volunteers in control and prevention efforts. However for effective control of Brucellosis in the present study area, an integrated approach should be promoted that takes into account the relationship between humans, animals and environment in the context of ‘‘One Health approach’’.

Competing interests

The authors’ declare that they have no competing interests.

Acknowledgements

We would like to extend our thanks to Kenya Field Epidemiology and Laboratory Program (K-FELTP) for financial support. We would also like to acknowledge the study

participants, the community leaders, County veterinary office in Garissa for support provided during the investigations.

References

1. Ducrotoy, M.J., et al., *Brucellosis as an Emerging Threat in Developing Economies: Lessons from Nigeria.* PLoS Neglected Tropical Diseases, 2014. **8**(7): p. e3008.

2. WHO, *Neglected zoonotic diseases.* 2014.

3. Pappas, G., et al., *The new global map of human Brucellosis.* The Lancet infectious diseases, 2006. **6**(2): p. 91-99.

4. Taleski, V., et al., *An overview of the epidemiology and epizootology of Brucellosis in selected countries of Central and Southeast Europe.* Veterinary microbiology, 2002. **90**(1): p. 147-155.

5. Seleem, M.N., S.M. Boyle, and N. Sriranganathan, *Brucellosis: a re-emerging zoonosis.* Veterinary microbiology, 2010. **140**(3): p. 392-398.

6. Radostits, O.M., et al., *Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. 2006: Elsevier Health Sciences.

7. Al-Majali, A.M., *Seroepidemiology of caprine Brucellosis in Jordan.* Small Ruminant Research, 2005. **58**(1): p. 13-18.

8. Queipo-Ortuño, M.I., et al., *Rapid diagnosis of human Brucellosis by peripheral-blood PCR assay.* Journal of clinical microbiology, 1997. **35**(11): p. 2927-2930.

9. Dean, A.S., et al., *Clinical manifestations of human Brucellosis: a systematic review and meta-analysis.* 2012.

10. Robinson, A. and A. Production, *Guidelines for coordinated human and animal Brucellosis surveillance*. 2003: FAO.

11. McDermott, J.J. and S. Arimi, *Brucellosis in sub-Saharan Africa: epidemiology, control and impact.* Veterinary microbiology, 2002. **90**(1): p. 111-134.

12. Kiambi, S.G., *Prevalence and factors associated with Brucellosis among febrile patients attending Ijara County Hospital, Kenya*. 2014.

13. Osoro, E.M., et al., *Strong Association Between Human and Animal Brucella Seropositivity in a Linked Study in Kenya, 2012–2013.* The American journal of tropical medicine and hygiene, 2015: p. 15-0113.

14. Smits, H., *Brucellosis in pastoral and confi ned livestock: prevention and vaccination.* REVUE SCIENTIFIQUE ET TECHNIQUE-OFFICE INTERNATIONAL DES EPIZOOTIES, 2014. **32**(1).

15. Schelling, E., et al., *Brucellosis and Q-fever sero-prevalences of nomadic pastoralists and their livestock in Chad.* Preventive veterinary medicine, 2003. **61**(4): p. 279-293.

16. County, G. *Garissa County Profile* 2013 08/08/2015]; Available from: www.garissa.go.ke.

17. KNBS. *Kenya Population and Housing Census*. 2009 [cited 2015 08/08/2015]; Available from: www.knbs.go.ke.

18. Thrusfield, M., *Veterinary epidemiology*. 2013: Elsevier.

19. Kansiime, C., et al., *Knowledge and perceptions of Brucellosis in the pastoral communities adjacent to Lake Mburo National Park, Uganda.* BMC public health, 2014. **14**(1): p. 242.

20. Holt, H.R., et al., *Brucella spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating sero-prevalence, risk factors and*

livestock owner's knowledge, attitudes and practices (KAPs). BMC Public Health, 2011. **11**(1): p. 341.

21. Grahn, C., *Brucellosis in small ruminants.* 2013.

22. Lindahl, E., et al., *A Study of Knowledge, Attitudes and Practices Relating to Brucellosis among Small-Scale Dairy Farmers in an Urban and Peri-Urban Area of Tajikistan.* PloS one, 2015. **10**(2).

23. Kang'Ethe, E., et al., *Investigations into the prevalence of bovine Brucellosis and the risk factors that predispose humans to infection among urban dairy and non-dairy farming households in Dagoretti Division, Nairobi, Kenya*. East African medical journal, 2007. **84**(11 Suppl): p. S96-100.

24. Buhari, H., et al., *Knowledge, attitude and practices of pastoralists on bovine Brucellosis in the north senatorial County of Kaduna state, Nigeria.* J. Anim. Health Prod, 2015. **3**(2): p. 28-34.

25. Adesokan, H.K., et al., *Knowledge and practices related to bovine Brucellosis transmission amongst livestock workers in Yewa, south-western Nigeria.* Journal of the South African Veterinary Association, 2013. **84**(1): p. 1-5.

26. Addo, K.K., et al., *Knowledge, Attitudes and Practices (KAP) of Herdsmen in Ghana with respect to Milk-borne Zoonotic Diseases and the Safe Handling of Milk.* J. Basic Appl. Sci. Res, 2011. **1**(10): p. 1566-1562.

27. Kozukeev, T.B., et al., *Risk factors for Brucellosis--Leylek and Kadamjay Countys, Batken Oblast, Kyrgyzstan.* 2003.

28. Earhart, K., et al., *Risk factors for Brucellosis in Samarqand Oblast, Uzbekistan.* International Journal of Infectious Diseases, 2009. **13**(6): p. 749-753.

29. Kozukeev, T.B., et al., *Risk factors for Brucellosis--Leylek and Kadamjay Countys, Batken Oblast, Kyrgyzstan, January-November, 2003.*

30. Laing, J., W. Morgan, and W. Wagner, *Brucellosis In: Fertility and infertility in veterinary practice.* English book language book society, Bailliere, Tindall, 1988: p. 189-220.

31. Megersa, B., et al., *Sero-prevalence of Brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia.* Trop Anim Health Prod, 2011. **43**: p. 651-656.

32. Boukary, A., et al. *Preliminary results of the study on zoonotic Brucellosis and tuberculosis in Niamey*. in *Globalization of tropical animal diseases and public health concerns. Proceedings of the 13th Association of Institutions for Tropical Veterinary Medicine (AITVM) Conference, Bangkok, Thailand, 23-26 August 2010*. 2010. Association of Institutions for Tropical Veterinary Medicine (AITVM).

33. Makita, K., et al., *Herd prevalence of bovine Brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda, Makita et al (2011).* BMC Veterinary Research, 2011. **7**: p. 60.

34. Mekonnen, H., S. Kalayou, and M. Kyule, *Serological survey of bovine Brucellosis in barka and arado breeds (Bos indicus) of Western Tigray, Ethiopia.* Preventive Veterinary Medicine, 2010. **94**(1): p. 28-35.

35. Muma, J., et al., *The role ofBrucellainfection in abortions among traditional cattle reared in proximity to wildlife on the Kafue flats of Zambia.* Revue Scientifique et Technique-Office International des Epizooties, 2014. **26**(3).

36. Sofian, M., et al., *Risk factors for human Brucellosis in Iran: a case–control study*. International Journal of Infectious Diseases, 2008. **12**(2): p. 157-161.

37. Regassa, G., et al., *Human Brucellosis in traditional pastoral communities in Ethiopia.* International Journal of Tropical Medicine, 2009. **4**(2): p. 59-64.

38. Poester, F., L. Samartino, and R. Santos, *Pathogenesis and pathobiology of Brucellosis in livestock.* Rev Sci Tech, 2013. **32**(32): p. 105-115.

39. Saegerman, C., et al., *Bovine Brucellosis.* Infectious and parasitic disease of livestock, 2010: p. 971-1001.

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

41. Kabagambe, E., et al., *Risk factors for Brucella seropositivity in goat herds in eastern and western Uganda.* Preventive Veterinary Medicine, 2001. **52**(2): p. 91-108.

42. Muma, J., et al., *Risk factors for Brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia.* Preventive veterinary medicine, 2007.

43. Al-Majali, A.M., et al., *Sero-prevalence and risk factors for bovine Brucellosis in Jordan.* Journal of Veterinary Science, 2009. **10**(1): p. 61-65.

44. Chand, P. and R. Chhabra, *Herd and individual animal prevalence of bovine Brucellosis with associated risk factors on dairy farms in Haryana and Punjab in India.* Tropical animal health and production, 2013. **45**(6): p. 1313-1319.

45. Chiebao, D., et al., *Variables Associated with Infections of Cattle by Brucella abortus., Leptospira spp. and Neospora spp. in Amazon Region in Brazil.* Transboundary and Emerging Diseases, 2013.

46. Patel, M., et al., *Prevalence and risk factor's analysis of bovine Brucellosis in peri-urban areas under intensive system of production in Gujarat, India.* World, 2014. **7**(7): p. 509-516.

Table 1: Distribution of demographic characteristics of respondents in Brucellosis knowledge, attitude and practices assessment, Garissa 2013

(n=120)

Variable	**Frequency** **n (%)**
Age group	
15-24	53 (44)
25-34	49 (41)
35-44	14 (12)
>45	4 (3)
Gender	
Male	90 (75)
Female	30 (25)
Education level	
None	92 (77)
Lower primary	25 (21)
Upper primary	3 (2)
Primary role in Herd	
Herd owner	58 (48)
Herding	38 (32)
Milking	24 (20)
Marital status	

Married	83 (69)
Single	37 (31)

Table 2: Distribution of responses of participants on awareness, cause and source of information on Brucellosis, Garissa 2013

(n= 95)

Variable	**Frequency** **n (%)**
Heard of Brucellosis□	
Yes	95 (79)
No	25 (21)
Cause of Brucellosis	
Don't know	38 (40)
Bacteria/germs	17 (18)
Food	14 (15)
Wild animals	13 (14)
Water	13 (14)
Source of information on Brucellosis	
Community Health Workers	44 (46)
Relatives/family member	19 (20)
Religious leaders	19 (20)
Veterinary staff	8 (9)
Local FM stations/media	5 (5)

☐**Subsequent analysis based on those who had heard of Brucellosis**

Table 3: Responses of participants on animal species affected by Brucellosis and signs and symptoms of Brucellosis in animals, Garissa 2013

(n= 95)

Variable	**Frequency** **n (%)**
Animal species affected*	
Goats	62 (65)
Sheep	47 (49)
Cattle	45 (47)
Camels	32 (34)
Signs and symptoms of Brucellosis*	
Abortions	56 (59)
Retained Placenta	21 (22)
Hygroma/Swollen joints	20 (21)
Swollen udder/Mastitis	11 (12)

***Multiple responses were permitted**

Table 4: Responses of participants on Brucellosis diagnosis and signs and symptoms in humans, Garissa 2013

(n= 95)

Variable	**Frequency** **n (%)**

Brucellosis diagnosis	
Family member diagnosed with Brucellosis in the past	46 (48)
Person not family member/relative diagnosed with Brucellosis	43 (45)
Respondent diagnosed with Brucellosis in the past	38 (40)
Signs and symptoms*	
Fever	71 (75)
Joint pains	56 (59)
Muscle pains	48 (51)
Loss of appetite	45 (47)
Chills	38 (40)
Headache	37 (39)
Night sweat	33 (35)
Fatigue	29 (31)
Malaise	29 (31)
Vomiting	14 (15)
Painful scrotum in men	14 (15)
Diarrhoea	12 (13)
Blurred vision	9 (10)
Miscarriage in women	7 (8)
Nausea	4 (5)

***Multiple responses were permitted**

Table 5: Responses of participants on attitude and perceptions towards Brucellosis, Garissa 2013 (n= 95)	
Attitudes and perceptions	**Frequency** **n (%)**
Attitude and Perception on Brucellosis seriousness	
Serious Disease in Animals	64 (67)
Serious Disease in Humans	61 (64)
Attitude and perception towards Brucellosis prevention in animals	
Brucellosis can be prevented in animals	13 (14)
Prevention by vaccination	6 (46)
Prevention by contacting veterinary office	4 (31)
Prevention by isolation of sick and aborting animals	3 (23)
Attitude and perceptions towards suspected human Brucellosis	
Brucellosis can be cured in humans	33 (35)
Seek Prayers	14 (42)
Visit health facility	8 (24)
Consuming herbal medicine	6 (18)
Visit local chemist and purchase medicine	5 (15)
Attitude and perceptions towards aborting animals	
Do nothing	44 (46)
Treat aborting animals with antibiotics	16 (17)
Sell the animal	11 (12)
Inform veterinary office	10 (11)
Isolate the animal	8 (8)
Slaughter the animal	5 (5)

Table 6: Responses of participants on practices towards Brucellosis, Garissa 2013 (n= 95)

Practices of respondents	**Frequency n (%)**
Consumption of raw milk	91 (96)
Assisted an animal during birthing/abortion/removal of retained placenta	72 (76)
Disposal of fetal materials	
Dumping	61 (75)
Burning	11 (25)
Burying	0 (0)
Use protective clothing	0 (0)
Participation in slaughtering/butchering an animal	34 (36)
Introduction of new animals into sheep and goat herd	46 (48)
Quarantine new animals	0 (0)
Lend male animals to other sheep and goat herds for breeding	30 (32)
Processing of raw milk products	13 (14)

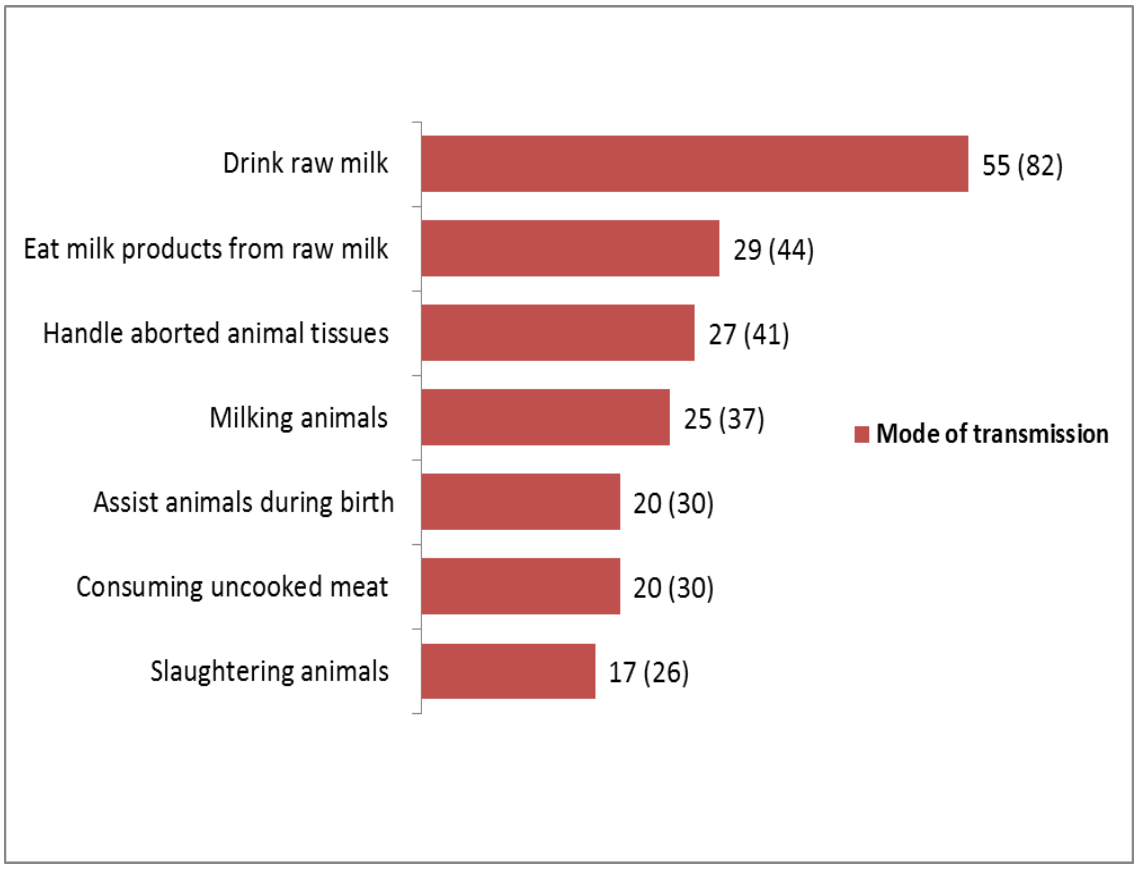

Figure 2: Responses of participants on mode of transmission of Brucellosis in humans, Garissa 2013 (n= 67)

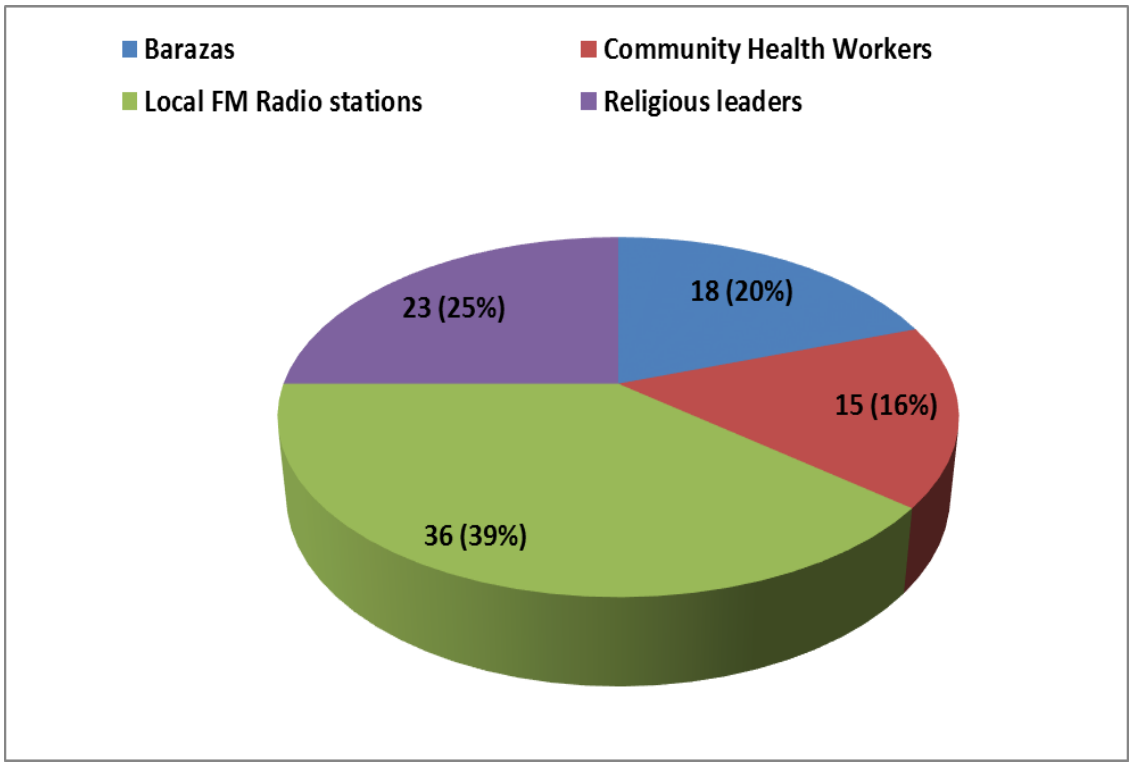

Figure 3: Responses of participants on preffered sources of more information on Brucellosis, Garissa County, 2013 (n= 95)