HOST-PATHOGEN ASSOCIATION AND TRANSMISSION DYNAMICS OF *BRUCELLA* SPECIES IN KENYA AND TANZANIA

BY

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DECLARATION

This thesis is my original work and has not been presented anywhere for a degree or any other award.

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DEDICATION

I dedicate this research to my wife; Anne, my sons; David and Henry, my daughter Angela, and my parents; David and Eunice.

ABSTRACT

Bacterial pathogen of the genus *Brucella* is a gram-negative organism with several species. It causes a disease called "brucellosis" in humans, and animals worldwide. Association between animal and human *Brucella* sero-positive cases have been documented within Kenya and Tanzania. But data on the species of *Brucella* circulating in various susceptible hosts, including the zoonotic species *B. abortus* and *B. melitensis* is insufficient, thus limiting adoption of species or host-targeted control strategies. The primary objective of this study was to assess host-pathogen association and transmission dynamics of *Brucella* species among animal and human populations. While the specific objectives were 1) To identify *Brucella* species circulating in livestock and wildlife in Marsabit, Narok in Kenya and Northern Tanzania 2) To identify *Brucella* species responsible for human infections in Marsabit, Narok in Kenya and Northern Tanzania 3) To assess molecular diversity and transmission dynamics of *Brucella* species in different hosts within Kenya and Tanzania. Therefore, a cross-sectional study was undertaken at the wildlife, livestock, and human areas in Marsabit and Narok, Kenya as well as Northern Tanzania. A total of 1384 samples from cattle (709), goats (274), sheep (191), pigs (79), camels (61), buffaloes (70), and humans (257) were collected from Narok, Marsabit in Kenya and Northern Tanzania. Information on history of retained placenta or abortion, location, and age of sampled livestock were recorded. Information such as gender, location of residence, and age were obtained and recorded from the study participants. Real-time PCR assays were run on all the samples with primers that are specific for IS711 and bcsp31 targets to detect the genus *Brucella*. Another real-time PCR assay with AlkB and BMEI1162 targets was run to detect *B. abortus* and *B. melitensis* species, respectively in all the samples that had amplifications with both genus targets to answer objectives one and two. Mixed effects logistic regression models and descriptive analysis were done using *lme4, sjstats,* and *gmodels* packages in R-statistical software. Overall, 199 (33.3%) livestock samples and 99 (38.5%) human samples were found to be positive for the genus *Brucella* for the third objective. This study found *B. abortus* to be associated with camels (OR=2.9, 95% CI: 1.3–6.3), and cattle (OR=2.3, 95% CI: 1.1–4.6), while *B. melitensis* had significant association with both goats (OR=1.7, 95% CI: 1.0 – 3.1) and sheep (OR=3.6, 95% CI: 2.0 – 6.7). The DNA belonging to both *B. melitensis* and *abortus* were found in multiple livestock species, buffaloes, and humans, suggesting occurrence of cross-transmission of the two *Brucella* species among the different hosts. Occurrence of cross-transmission of *Brucella* spp. beyond their known preferential host was further strengthened by detection of *B. abortus* in Pigs, that has always been associated with *B. suis.* Animals with either retained placenta or abortion history were associated with presence of *B. melitensis*, and *B. abortus*, respectively. Therefore, retained placenta and aborted materials could be facilitating the transmission of *B. melitensis* and *B. abortus*. Persons within 21 to 40 years of age were more likely to have a PCR positive results for *Brucella* (OR=2.8, 95% CI: 1.2-6.6) than other age categories. Multiple livestock species are responsible for transmitting *Brucella* to humans. Therefore, brucellosis control in humans should target cattle, sheep, goats, pigs, and camels, while using One Health multidisciplinary approach.

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LIST OF ABBREVIATION AND ACRONYMS

ANOVA	Analysis of Variance
CDC	Centre for Disease Control and Prevention
CI	Confidence Interval
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FBTA	Febrile Brucella Agglutination Test
AICUC	Institutional Animal Care and Use Committee
ILRI	International Livestock Research Institute
MLVA	Multiple Locus Variable Number Tandem Repeat Analysis
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
RBT	Rose Bengal Test
SSA	Sub – Saharan Africa
UNESCO	United Nations Education, Science and Cultural Organization
WHO	World Health Organization

DEFINITION OF TERMS

Biovar - A variant prokaryotic strain that differs physiologically and /biochemically from other strains in a particular species

Prevalence-The proportion of individuals in a population having a disease over the total at a given time

Primer- Short DNA segment complementary to the template

Zoonotic disease- Diseases that can spread between vertebrate animals and people

Transmission dynamics- Mode or methods that a pathogen uses to move amongst hosts

Abortion- Expulsion of a fetus from the uterus before a stage of viability

Molecular diversity- Variation in distribution of *Brucella* species in various hosts and locations

Number of diseased- Estimated number of diseased animals or people within a **Population.** This is achieved by multiplying the total population (N) with expected prevalence

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CHAPTER ONE
INTRODUCTION

1.1 Background Information

Brucella is a genus of a gram negative bacterial organism, with several species, namely: *B. abortus, B. ovis, B. melitensis, B. canis, B. suis, B. neotomae*, affecting several animals and humans worldwide (D'Anastasio et al., 2011). Whereas, *B. pinnipedialis*, and *B. ceti*, were recently detected in marine animals (D'Anastasio et al., 2011). In terms of pathogenicity to humans, *B. melitensis* is regarded as the most pathogenic species, followed by *B. suis*, *B. abortus* and *B. canis* (Hadush, 2013). Both *B. abortus* and *B. melitensis* have been reported to be circulating in cattle in Isiolo as well as Central and Eastern parts of Kenya (Muendo et al., 2012). Similarly *B. abortus* was detected in cattle in Southern parts of Tanzania (Mathew et al., 2015), while *B. abortus* and *B. melitensis* reported in humans from the Northern parts of Tanzania (Bodenham et al., 2020; Mathew et al., 2015; Muendo et al., 2012; Wainaina et al., 2020).

However, no reports of *B. suis* or *B. canis* has been recorded in both humans and animals within Kenya and Tanzania, suggesting low or non-circulation of *B. suis* and *B. canis* in the region. Brucellosis in animals results in huge economic losses as a result of reproductive disorders such as abortion, infertility, and low milk yields in livestock, while humans tend to suffer from a chronic and debilitating illness, associated with undulating fever (Megid et al., 2010). Brucellosis occurrence in humans is largely due to transmission from infected animals through consumption of raw or undercooked contaminated animal products, especially milk or through direct or indirect contact with secretions from sick animals, especially during the parturition period (Corbel & Goonaratna, 2006). *Brucella* bacteria are also highly infectious, thus posing work-related risk to persons dealing with products from *Brucella* infected animals, including laboratory personnel, slaughter personnel and farmers who assist animals when giving birth (Hadush, 2013).

Brucellosis is considered as one of the most common zoonotic diseases in the world, with an annual report of more than 500,000 new human cases, mainly coming from the developing countries (Chen et al., 2013; Skalsky et al., 2008). It causes considerable production losses in cattle, sheep, goats, and camel populations, predominantly among the pastoral communities and

the extensive agro-pastoral systems in sub-Saharan Africa (SSA), where humans reportedly acquire *Brucella* infection regularly (McDermott & Arimi, 2002). Brucellosis also occurs as an emerging and re-emerging problem in intensified peri-urban small holder dairy production (Ducrotoy et al., 2015; Njeru et al., 2016).

While several serological studies conducted in livestock, wildlife and humans in many parts of sub-Saharan Africa revealed that *Brucella* antigens or antibodies are widespread, they are incapable of identifying the species of *Brucella* responsible for the production of antibodies in the host (Godfroid et al., 2011, 2013). Few fragmented studies in sub-Saharan Africa have isolated *Brucella* species (Assenga et al., 2015; Mathew et al., 2015; Muendo et al., 2012; Skalsky et al., 2008), but they have not adequately provided information related to the various *Brucella* species that are circulating in the different susceptible hosts in SSA. Instead, the documented data indicate that there is cross-infection of different *Brucella* species within the same host. For instance, *B. melitensis,* and *B. abortus* have reportedly been detected in cattle together (Muendo et al., 2012). Similarly, these same bacterial species, as well as *B. ovis,* have been detected in sheep and goats (Ducrotoy et al., 2015; McDermott & Arimi, 2002). In a separate study, one *Brucella* species (*B. abortus*) was isolated from cattle, and horses (Ocholi et al., 2004), furthermore, *B. melitensis* was also recently detected in camels (Muturi et al., 2021) thus, complicating the current knowledge on the preferential nature of *Brucella* species, with regards to different susceptible animal host in sub-Saharan Africa. The few studies conducted in Kenya and Tanzania focused on cattle and goats, with only one study documenting *Brucella* spp. in camels. Information on circulating species of *Brucella* in pigs and sheep were not available. Therefore, this study was conducted to identify *Brucella* species and their association with various livestock and wildlife hosts in Northern Tanzania, as well as Narok and Marsabit Counties, Kenya. These target regions are areas with high numbers of livestock and wild animals, but with insufficient information on the species of *Brucella* responsible for the sero-positive cases previously detected in these animals.

The non-specific nature of clinical presentation and the diversity of differentials for brucellosis, especially in humans often leads to misdiagnosis and delayed treatment. The wide range of signs and symptoms are similar to those presented by common tropical diseases like typhoid and malaria (Muriuki et al., 1994). One study in Kenya, confirmed that 81.5% of brucellosis cases

were not detected through clinical examination in a health facility, but treated for malaria or typhoid, and that 2.7% of samples sent to the laboratory were seronegative, but later turned out positive on real-time PCR (Njeru et al., 2016). Similarly, brucellosis has been recognised as a contributor to the underdiagnosed and untreated febrile cases among adult and paediatric patients hospitalised in northern Tanzania (Bouley et al., 2012). However, in a rather contrasting finding from a different study, human brucellosis was found to be overestimated using the Febrile *Brucella* Agglutination Test (FBAT) (de Glanville et al., 2017). The World Health Organization currently recommend the Rose Bengal Test for the rapid screening of brucellosis, Enzyme-Linked Immunosorbent Assay (ELISA) for confirmatory and bacteriological culture as the gold standard test (World Health Organization, 2006). In Kenya, the first case of brucellosis in human was detected in 1916, and since then, documented studies suggest high sero-prevalence (14.4-46.5%) in people living in pastoral and agro-pastoral areas (7.0-15.3%), with the highest (46.5%) being in Marsabit, while low levels (0.1-2.4%) have been reported in individuals from small scale livestock holder regions (McDermott & Arimi, 2002; Njeru et al., 2016; Omballa et al., 2016; Osoro et al., 2015). One of the serological studies done in Marsabit County, Kenya found that human brucellosis is likely linked to brucellosis cases in livestock (Osoro et al., 2015). However, identification of *Brucella* species in a linked animal-human study is yet to be done. Therefore, identification of species of *Brucella* responsible for human infection remains a fundamental gap in understanding the epidemiology of *Brucella*, especially in areas where there is frequent contact between animals and humans such as Marsabit, Narok, and Northern Tanzania. As a result, this study was done to identify species of *Brucella* circulating in the animal populations and compare with those in humans to inform animals to be targeted in brucellosis control in humans.

Data from serological studies conducted in Tanzania suggests that close contact between human-livestock and wildlife facilitates the maintenance of brucellosis in an ecosystem (Assenga et al., 2015; Kunda et al., 2010; Shirima et al., 2010; Shirima & Kunda., 2016). Similarly, indication of association between human and livestock seropositive cases within the same household was recorded in Kenya (Osoro et al., 2015). Despite the serological indication, information on the distribution of *Brucella* species across the different geographical locations as well as their distribution in human, wildlife and livestock hosts remains scarce, with very limited efforts of molecular typing of *Brucella* to identify the species and biovars in circulation to explain the host-

pathogen association and transmission dynamics. Thus, this study was conducted to identify circulating *Brucella* spp. and dynamics of their transmission in the different hosts within the wildlife, livestock, and human ecosystems of Marsabit, Narok and Northern Tanzania.

This study investigated *Brucella* species circulating in both humans and livestock. Therefore, only zoonotic species of *Brucella* were investigated. Humans are mostly affected by *B. abortus*, *B. melitensis*, and *B. suis* (OIE, 2016). All the studies conducted in Kenya and Tanzania have reported the presence of both *B. abortus* and *B. melitensis* as the main circulating species of *Brucella* in the two countries (Bodenham et al., 2020; Mahlau, 1967; Mathew et al., 2015; Muendo et al., 2012; Ntirandekura et al., 2020; Oomen, 1976; Philpott & Auko, 1972; Wainaina et al., 2020). However, no reports of *B. suis* has been recorded in both humans and animals within the Kenya and Tanzania, suggesting low or non-circulation of *B. suis* in the region. An attempt to detect *B. suis* in the camel population failed to get any positive case (Muturi et al., 2021). My attempt to also detect *B. suis* from wildlife samples did not find any positives. Therefore, the study relied on existing knowledge to only focus on the two species of *Brucella* that are recognized for their public health importance in the region.

1.2 Problem Statement

Brucellosis has been effectively controlled in populations of livestock in several developed nations using species-specific livestock vaccines and subsequent test and slaughter. Yet, remains endemic, but neglected zoonosis in many parts of the world, with over 500,000 new human brucellosis cases being documented annually, especially from Africa and Asia. Several serological studies have demonstrated that brucellosis is endemic in Kenya and Tanzania. But data on *Brucella* spp. circulating in various animal hosts is insufficient, with the few studies only focusing on cattle and goats, while none documenting circulating species of *Brucella* in sheep and pigs. Thus, limiting adoption of species or host-targeted control strategies, while using control strategies such as vaccination of animals. Similarly, only few studies have investigated *Brucella* species in human in Kenya and Tanzania. Thus, the knowledge of circulating *Brucella* spp. in human population is insufficient and the contribution of different animal species to human brucellosis remains unclear. Therefore, this study bridges this knowledge gap through identification of *Brucella* species that are responsible for infection in different animal hosts and humans.

While significant association between animal and human *Brucella* sero-positive cases have been recorded in Kenya and Tanzania, animal species with responsible for the highest contribution to the transmission of *Brucella* to human is poorly known. Additionally, how the transmission takes place between different susceptible hosts in wildlife, livestock, and human ecosystems, such as Marsabit, Narok and Northern Tanzania remains unclear. A modelling run on serological data sets from Northern Tanzania to establish source of infection in human, hypothesized that goats and sheep are the major contributors to human infection. Another finding from Marsabit was that persons living in households with sero-positive animals were six times more likely to contract *Brucella*, especially if the sero-positive animals were camels or goats. However, studies aimed at identifying the species of *Brucella* from infected animal types and humans who are exposed to such animals has not been done. Thus, the knowledge on association between the different animal types with specific *Brucella* species and subsequent transmission dynamics to humans and between various susceptible animal hosts is insufficient. Therefore, this study was done to identify *Brucella* species responsible for infection in the different animal hosts and humans, as well as establish their transmission dynamics.

1.3 Objectives

1.3.1 General Objective

To assess *Brucella* host-pathogen association and transmission dynamics among animal populations and humans in Kenya and Tanzania.

1.3.2 Specific Objectives

i. To identify *Brucella* species infecting livestock and wildlife in Marsabit, Narok in Kenya and Northern Tanzania.

ii. To identify the species of *Brucella* responsible for human infections in Marsabit and Narok, Kenya.

iii. To assess molecular diversity and transmission dynamics of *Brucella* species in different hosts wildlife, livestock and human hosts within Kenya and Tanzania.

1.3.3 Research Questions

i. What are the species of *Brucella* responsible for infecting livestock and wildlife in Marsabit, Narok in Kenya and Northern Tanzania?

ii. What are the species of *Brucella* responsible for human infections in Marsabit and Narok, Kenya?

iii. What are the molecular diversity and transmission dynamics of *Brucella* in different wildlife, livestock and human hosts within Kenya and Tanzania?

1.4 Significance of the study

Data available from Kenya and Tanzania on the genotypic characterization of *Brucella* species has mainly been generated using samples from human and cattle. Therefore, information on the species of *Brucella that* are circulating in other susceptible animals remained unknown. This study has addressed the knowledge gap by targeting all susceptible livestock hosts and generated information on the circulating species of *Brucella* in pigs, goats, cattle, sheep, camels, as well as the wildlife.

This study also detected both *B. abortus* and *B. melitensis* to be circulating in the human population. The two species (*B. abortus* and *B. melitensis*) were found to be circulating in almost equal proportions. Therefore, suggesting that multiple livestock species including cattle, sheep, goats, camels, and pigs could be involved in the transmission of *Brucella* to humans. This finding provide a basis for development of policies and control programs that target multiple livestock species in brucellosis control programs to effectively control brucellosis in humans and contribute to good health and wellbeing, as outlined in the third Sustainable Development Goals (SDGs) of the United Nations.

Existing associations between *B. abortus* and *B. melitensis* with the different animal hosts was established in this study. Furthermore, research findings point to potential occurrence of cross transmission of *Brucella* species between the different susceptible host. Therefore, contact between different animal species should be reduced to avoid cross transmission of different *Brucella* species. The presence of *B. abortus* and *B. melitensis* was associated both history of abortion and retained placenta in animals. Thus, providing evidence that proper disposal and handling of aborted materials and retained placental tissues should be considered as one of the ways of reducing the risk of transmitting *Brucella* spp.

CHAPTER TWO

LITERATURE REVIEW

2.1 Identification of *Brucella* Species Circulating in Animals

2.1.1 Livestock

Brucella survives and replicates inside phagocytic cells of the host, it evades and modulates immunological responses, and moves to the tissues of preference such as fetal lungs, the trophoblasts in pregnant females, as well as reproductive and reticuloendothelial systems (De Figueiredo et al., 2015). A four-carbon polyol, known as erythritol in fetal tissues of ruminants is important in colonization of the uterus, it stimulates major *Brucella* virulence pathways (Barbier et al., 2017; Poesster et al., 2013). High levels of erythritol have been demonstrated to stimulate the growth of *B. abortus* and *B. melitensis* (Hamer et al., 2014; Petersen et al., 2009). Abortion results from acute reproductive tract pathology as a result of widespread replication of *Brucella* in the placental trophoblasts, while chronic infection occur because of persistence within the macrophages (Salcedo et al., 2013). Taxonomically, the *Brucella* genus is a member of the Brucellaceae family, of the order Rhizobiales and the α-2 subdivision of *proteobacteria.* It is a non-spore-forming, gram negative, coccobacillus organism. With short rods, partially acid fast, it utilizes virulence factors, such as the VirB type IV secretion system to inhibit the host's cells defenses to create a suitable intracellular niche to support its survival and subsequent replication within the host macrophages (O'Callaghan & Whatmore, 2011). The biochemical characteristic of *Brucella* indicate that it is nitrate reductase, catalase, urease, and oxidase positive (Akhvlediani et al., 2010). Described in Table 2.1 are the 10 known species, their preferred hosts and respective serotypes (Whatmore, 2009; Xavier et al., 2010).

Table 2.1: *Brucella* species, biovars and preferred hosts as well as pathogenicity in humans

Species	Biovars	Preferred hosts	Pathogenicity in humans
Classical Species			
B. melitensis	1,2,3	Sheep, goats, camels	High
B. abortus	1-6, 7, 9	Cattle, bison	High
B. suis	1 ,3	Pigs	High
	2	Wild boar, hare	Low
	4	Reindeer, caribou	High
	5	Rodent	No
B. canis		Dogs	moderate
B. neotomae		Desert rat	moderate
B. ovis		Sheep	No
Newly identified species			
B. pinnipedialis		Seals	Mild
B. ceti		Cetaceans	Mild
B. microti		Voles	No
B. inopinata		Unknown	Unknown

Brucella species can be identified by isolation in animals using selective or plain media to grow Brucella culture from blood, uterine discharges, udder secretions, aborted fetuses, or selected tissues, like swabs, from reproductive system and lymph nodes. Culture and isolation offers the definitive diagnosis of brucellosis in both animals and humans. However, the use of cultures is limited by its laborious and time consuming nature, as well as biohazardous (Corbel & Goonaratna, 2006).

The molecular biological techniques for typing *Brucella* spp. are based on the polymerase chain reaction (PCR) amplification using extracted DNA (with commercial kits) (Smirnova et al., 2013; Yu & Nielsen, 2010). Molecular methods are faster and provide more accurate reproducible results than the conventional culture and immunological essays (Saini et al., 2017). Molecular diagnostics can detect small amounts of bacterial DNA in individuals undergoing antibacterial therapy and give both complementary and bio-typing methods that are based on the specific genomic sequences. The real-time PCR assays, like those established by Probert and Matero (Matero et al., 2011; Probert et al., 2004), have contributed to significant increase and ease of detecting *Brucella* DNA, especially, using the genomic materials that are extracted directly from clinical specimens. These test possibilities and results have improved the understanding on the intricate epidemiology of *Brucella* between diverse hosts. The Real-time PCR offers better specificity , sensitivity, and speed of operation, in comparison to conventional

PCR (Alamian et al., 2017). However, sensitivities and specificities may vary due to prevailing factors and absence of standardization (Wang et al., 2014).

2.1.2 Different Wildlife Hosts

Antibodies of *Brucella* spp. have consistently been detected in several wildlife species, including blue wildebeest, African buffalo, zebra, and impala(Alexander et al., 2012; De Vos & Van Niekerk, 1969; Ducrotoy et al., 2015; Herr & Marshall, 1981). Indications are that brucellosis in wildlife is commonly detected in gregarious animals like wildebeest, buffaloes and impalas, as opposed to solitary animals like rhinoceros (Ducrotoy et al., 2017; Simpson et al., 2021). Interaction between livestock and wildlife, in is regarded as a key risk factor for transmission of *Brucella* in wildlife, it is alleged that wildlife often acquire *Brucella* spp. from livestock (Ducrotoy et al., 2017). However, there are cases where some *Brucella* spp. perpetuate within the wildlife population without contact with livestock (Ducrotoy et al., 2017). This was demonstrated by a study conducted in Zambia, that found that *Brucella* infection could have been circulating in buffaloes, independently and without any interactions with cattle (Motsi et al., 2013).

There are indications that cross-transmission of *Brucella* occurs between wildlife and livestock. This was supported by the detection of higher sero-prevalence rate in livestock from wildlife-livestock interface areas (20.4%), as opposed to livestock from non-interface areas (13.45%) (Motsi et al., 2013) and (Enström et al., 2017)Similarly, cattle that grazed within the park had a higher ($p<0.001$) sero-prevalence (13.5%) in relation to those without any history of being grazed with the park (4.9%) (Gomo et al., 2012). Therefore, surveillance for brucellosis in wildlife, livestock, and people, involving molecular analysis of *Brucella* species and biovars should be conducted in areas with wildlife, livestock and human interactions to fully explore dynamics of transmission of *Brucella* spp. and the role of wildlife in *Brucella* transmission.

In sub-Saharan Africa, *Brucella* spp. involved in wildlife infection and range of susceptible wildlife hosts have not been fully established, even though the presence of *B. abortus* biovar 1 in buffalo, eland and waterbuck has been documented (Ducrotoy et al., 2015). This study detected and characterized *Brucella* species with the objective of refining the understanding on the molecular epidemiology of *Brucella spp.* in wild animals.

2.2 Identification of *Brucella* spp. in Human

Infection in humans are mostly caused by *B. melitensis, B. abortus, B. suis* and, in rare cases, *B. canis*. *Brucella* have been listed as a notifiable organism by OIE (OIE, 2016), and a priority pathogen by the US Center for Disease Control and WHO, mainly due to the high virulence of *B. suis* and *B. melitensis* strains that were previously experimentally developed to be used as a biological weapon due to their comparative stability in aerosol and the low dose required for infection to occur (Goonaratna, 2009; World Health Organization, 2006). Knowing the main species of *Brucella* responsible for causing clinical cases in human population is important in identifying the animal hosts responsible for zoonotic transmission and thus forms a very important step in developing targeted control program.

Brucellosis in humans is characterized by non-specific signs that resemble certain common tropical diseases, including typhoid fever and malaria (Franc et al., 2018). These symptoms include joint pains, malaise, musculoskeletal pains, body wasting, fever, sweating, and headache. Due to the rather difficult differential diagnosis, it is commonly misdiagnosed as malaria, and mostly laboratory diagnosis is done only after the initial failure by the patient to respond to malaria treatment. Notwithstanding, several diagnostic tests are available, such as culture and isolation of *Brucella* spp. that is considered as definitive test for diagnosis of brucellosis, especially on acute cases (Corbel & Goonaratna, 2006). The Rose Bengal Test and ELISA procedures are recommended as useful serological essays (Goonaratna, 2009). Techniques for amplification of nucleic acid have also been developed for quick detection in addition to confirmation of *Brucella* species and strains (Probert et al., 2004).

Human brucellosis is well-documented through several serological studies previously conducted in both Kenya (Alumasa et al., 2021; Fèvre et al., 2017; Kairu-Wanyoike et al., 2019; Muriuki et al., 1994; Njeru et al., 2016; Osoro et al., 2015) and Tanzania (Bouley et al., 2012; Crump et al., 2013; Kunda et al., 2007; Lukambagire et al., 2021). However, phenotypic, and genotypic data remains scarce in both regions. Therefore, this study would generate information on *Brucella* species circulating in human populations in Marsabit, Narok and Northern Tanzania.

2.3 Molecular Diversity of *Brucella* in different Wildlife, Livestock, and Human Hosts

Even though distinct species within the genus *Brucella*, have been identified alongside varied pathogenesis and host preferences, the DNA-DNA hybridization studies have revealed that the

genus *Brucella* is a homogenous genetical group, with all species sharing 90% of DNA homology (Cloeckaert et al., 1996; Verger et al., 1985; Whatmore, 2009). The rapid advancement of new technologies have led to improved understanding of *Brucella* diversity (Whatmore, 2009). Differential tests that are based on antigen typing, dye sensitivity, phenotypic characterization of lipopolysaccharide antigens, H2S production, metabolic properties and CO2 requirement, are used to distinguish *Brucella* species and biovars (Cloeckaert et al., 1996). A lot of research was previously focused on differentiating the members of the genus *Brucella* through identification of molecular markers. However, species and sub-species identification offers more detailed information for epidemiological traceback and development of species-specific elimination programs.

Analysis of the variable number of tandem repeats (VNTR) has previously been utilized to discriminate *Brucella* spp. despite the existence of minimal genomic diversity (Vergnaud & Pourcel, 2014). A recent cluster analysis using VNTR demonstrated that cluster groups correspond to the conventional spp. designations (Whatmore et al., 2007). In addition, cluster correlating to *B. abortus, B. ovis, B. melitensis,* and *B. neotomae* were recognized together with *B. suis* cluster and sub-clusters corresponding to biovars 1 and biovars 2, 4 and 3, while *B. suis* biovars 1, 3 and 4 were identified as closely related to *B. canis*, whereas *B. suis* biovar 5 appeared to be different from the rest of the *suis* biovars (Whatmore, 2009), Figure 2.1.

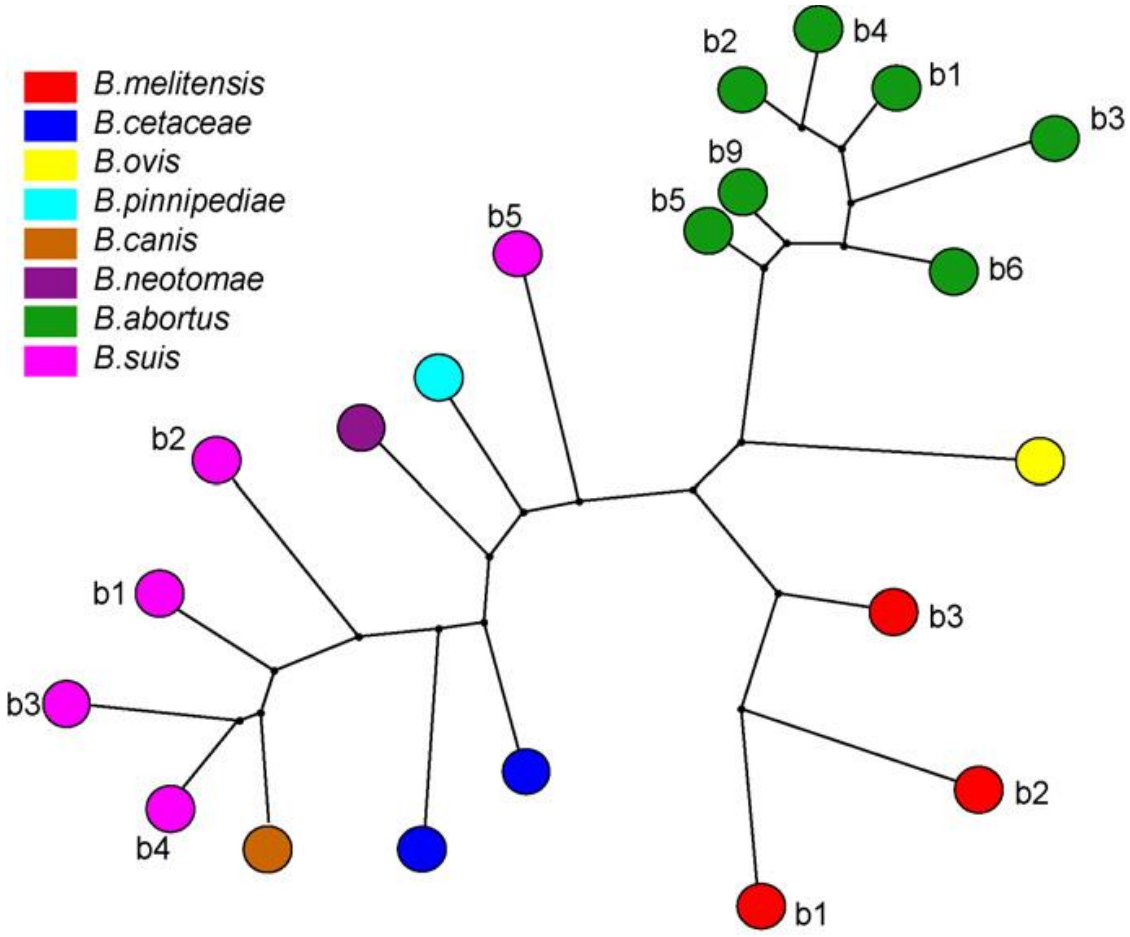

Figure 2.1: VNTR cluster analysis of *Brucella spp*. and biovars

The few studies previously conducted in Kenya and Tanzania identified *B. melitensis* biovar 1,2 & 3 (Mahlau, 1967; Oomen, 1976) and *B. abortus* biovar 1 & 3 (Assenga et al., 2015; Mathew et al., 2015; Muendo et al., 2012), to be present in the region. However, they are not adequate to fully explain the diversity of *Brucella* spp. in the different hosts within the study region. As such, the current study identified zoonotic species of *Brucella* circulating in different hosts including sheep, cattle, goats, pigs, camels, buffaloes, and humans, in addition to establishment of the transmission dynamics of *Brucella* spp. within Kenya and Tanzania.

CHAPTER THREE
METHODOLOGY

3.1 Study Area

Narok, Kenya and Northern Tanzania were selected for the human and livestock components (except pigs) based on the high numbers of both wild animals and livestock populations in close contact with humans within the same ecosystem. This creates a conducive environment for a zoonotic pathogen like *Brucella* to be transmitted. Marsabit County in Kenya was selected because it has the highest record of human and camel brucellosis prevalence in the East Africa region (McDermott & Arimi, 2002; Njeru et al., 2016; Osoro et al., 2015). Below is the map showing the study area, where cattle, sheep, goats, camels, and humans were sourced (Figure 3.1). Pig samples were obtained from the pig keeping areas in Kenya, given that the pigs were not kept in the selected study sites within Narok, and Marsabit in Kenya (Njanja et al., 2003; Nthiwa et al., 2019a).

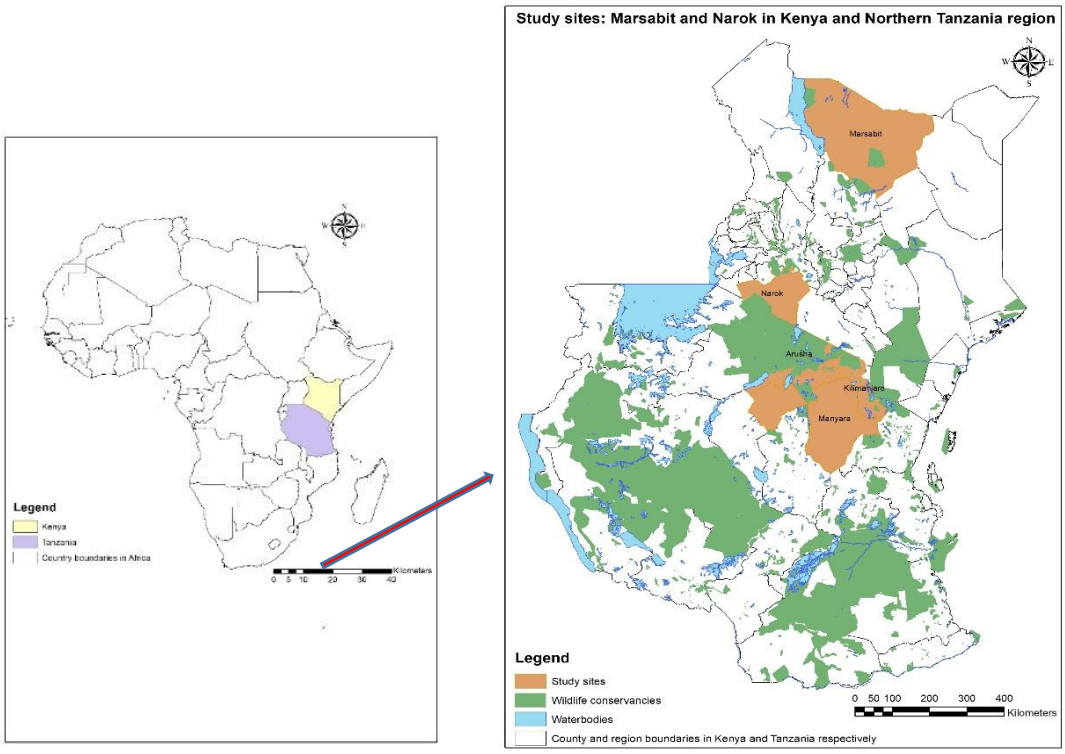

Figure 3. 1 Location of Kenya and Tanzania in Africa (left) and the study sites within Kenya and Tanzania (right).

3.1.1 Narok County

Narok County is located on the southern border of Kenya with Tanzania, it has an altitude of 1700-3000 meters above the sea-level. It is a predominantly pastoral area with wildlife tourism and wheat farming being the major economic activities (Muriuki et al., 1994). Narok County is home to the Maasai Mara national park that is well endowed with high numbers of different wild animals. Among them are those that have previously been recorded susceptibility to *Brucella* spp. including; eland, oryx, buffaloes, impala, wildebeest, zebra, waterbuck, elephant, giraffe, hartebeest, kudus, rhino, warthog, bushbuck, honey badger, and lion among others (Alexander et al., 2012; Ducrotoy et al., 2015; Mathew et al., 2015; Paling et al., 1988). Maasai Mara is an expansion of northern Serengeti game reserve, which is a very important wildlife migration path between Mara region in the Republic of Tanzania, and Kenya. Thousands of wildebeest, Thomson's gazelle and zebra migrate annually from Serengeti into Maasai Mara and back, from the month of July through to October. The latest livestock census in Kenya, ranked Narok as the county with the second highest number of cattle, and it is among the top seven leading in populations of goats and sheep as well as dairy production (cattle population 1,416,886, sheep 1,650,029 and goats 880,218) (KNBS, 2014).

The Maasai community inhabiting this area mainly practices pastoral and agro-pastoral livestock management, with frequent sharing of grazing areas with wild animals. Looking at this from a biomedical perspective, people staying in close contact with either livestock, wildlife, or both and also those consuming contaminated animal-sourced food intensifies their risk of getting exposed to different zoonotic pathogens such as *Brucella* spp. The high population of *Brucella* susceptible hosts, both wild and domesticated animals that live in proximity to humans, makes Narok County within the Maasai-Mara ecosystem an ideal site to investigate *Brucella* host pathogen associations and transmission dynamics. Moreover, interaction between different livestock species, wildlife, and humans has been reported as common in the neighboring agro-pastoral and pastoral communities, living within Tanzania (Assenga et al., 2015). A recent study in Narok County further established that seropositivity of brucellosis higher in livestock that are kept closer to the wildlife conservation areas as opposed to these kept far away from the wildlife (Nthiwa et al., 2019b).

3.1.2 Marsabit, Kenya

Marsabit is the largest, most arid, and least inhabited region in Kenya, with a vast lowland scrub desert ranging from 400m to 700m latitude, and an annual rainfall of 200mm in the lowlands and 1000mm in the highlands. Nomadic pastoralism that involves mixed herds of camels, cattle sheep and goats is the main economic activity, accounting for up to 80% of the occupants (Elliot et al. 2006). Therefore, majority of people in Marsabit stay in close proximity to their animals and commonly practice some of the known risky behaviors for transmission of *Brucella* such as the use of a common grazing and drinking areas for livestock, nomadic movements and drinking of raw milk (Osoro et al., 2015). Furthermore, the findings from a recent brucellosis study in Marsabit not only recorded a sero-prevalence of 46.5% in humans, which is the highest ever reported in Kenya and the entire East African region, but also found that human sero-positivity was six times more in households that had at least one sero-positive animal as opposed to those without (McDermott & Arimi, 2002; Njeru et al., 2016; Osoro et al., 2015; Wareth et al., 2016). Despite known exposure of camels, cattle, sheep, and human, to brucellosis in Marsabit, collection of information on the species of *Brucella* infecting the different hosts and their transmission dynamics is yet to be done. This made it a suitable study site for the current study.

3.1.3 Northern Tanzania

The northern Tanzania region is one of the renowned wildlife areas in the world, that hosts the famous Serengeti, Kilimanjaro, Lake Manyara, and Tarangire National parks, all recognized as UNESCO World Heritage sites. Ngorongoro is another important wildlife conservation park in Northern Tanzania (Rodgers et al., 2003). The wildlife in this region has retained the diversity and abundance, with some unique annual movements across the wider landscape and between different ecosystems and wildlife ranges, as well as privately owned and communal lands (Rodgers et al., 2003). This extended movements of wildlife into privately owned and communal grazing lands facilitates close interaction between livestock, wildlife and humans and making zoonoses and other animal-associated foodborne diseases important.

Additionally, Northern Tanzania is characterized by both pastoralism and small holder systems, with mixed crop -livestock farming, such as in the Kilimanjaro region (Bouley et al., 2012). The combined livestock population for Manyara, Kilimanjaro and Arusha regions of Northern Tanzania were estimated as 3,970,224 cattle, 2,398,516 sheep and 3,942,201 goats (Livestock

2013). It is therefore possible to compare the *Brucella* species circulating in the different production systems. Mobile pastoralism has been reported to enhance transmission of infectious diseases, and that brucellosis is more common in pastoral areas (Cox & Sharp, 1999; Dinka & Regassa, 2009). Arusha and Manyara areas within the Northern Tanzania that host the majority of mobile livestock communities in the entire Republic of Tanzania, whereby the key communities are Maasai and Barbaigs, who predominantly practice traditional mobile pastoral livestock keeping (Kunda et al., 2007). Therefore, Northern Tanzania was an ideal site for investigating *Brucella* species and transmission dynamics between the various susceptible hosts.

3.1.4 Study site for the pig population

Pigs are not uniformly distributed, but clustered in different areas across Kenya and Tanzania, and were not kept in Narok, and Marsabit in Kenya (Njanja et al., 2003; Nthiwa et al., 2019a). Therefore, samples from the pig keeping regions in Kenya and Tanzania were targeted. Archived samples previously collected at the largest pig abattoir in Nairobi was obtained. The abattoir is situated within the suburbs of Nairobi and is supplied with pigs from all the pig keeping regions in Kenya (Akoko et al., 2019). Similarly, pig samples were also obtained through a study that collected samples from the pig keeping areas in Tanzania.

3.2 Study design

This study used a cross-sectional study design, with a risk-based sampling approach. This involves selection of study participants with the highest probability of being exposed to a disease (Stärk et al., 2006). Disease symptoms and known risk factors for transmission are used to identify participants with the highest chance of being exposed or positive for a disease in a population. The risk-based study design was adopted in this study to increase probability of finding animals with circulating *Brucella* DNA to be used for identification of *Brucella* species.

3.3 Study population

The study population comprised of cattle, sheep, goats, and camels kept in Marsabit, Narok, within Kenya and the Northern Tanzania regions and people living in these areas, with the following categories targeted for inclusion.

i. Livestock populations that have previously been identified to have a higher incidence of brucellosis based on previous studies or clinical history were targeted.

ii. Pigs from any part of Kenya and Tanzania were targeted for the study, given that they are not kept in Narok and Marsabit, Kenya and Northern Tanzania.

iii. Samples collected from buffaloes in Marsabit, Narok and the neighboring counties were targeted for inclusion.

iv. Human population: persons seeking medication within selected health facilities in the livestock sampled areas were targeted for the human component of the study. The study population involved all patients referred to laboratories for brucellosis testing, based on clinical suspicion, and provided an informed consent to be included in the study.

3.4 Inclusion and Exclusion Criteria

3.4.1 Livestock

Inclusion Criteria

i. Cattle, sheep, goats, pigs, and camels were included in the study

ii. Animal herds with suspected history or case of brucellosis within the past 1 years. Adult animals within the herd were selected, with priority being given to animals with history of abortion or retained placenta infertility, and actively serving males.

Exclusion criteria

a) Very weak animals due to disease or injury.

3.4.2 Wildlife

Inclusion criteria

i. Only buffalo samples were included in the study. Buffaloes were targeted due to a relatively higher prevalence rate of *Brucella* antibodies that have been documented in buffaloes in comparison to the rest of wildlife species.

ii. Archived buffalo samples collected between the year 2001 and 2021.

iii. Any sample with information on the species of animal.

Exclusion criteria

a) Sample without any records, especially animal species.

3.4.3 Human

Inclusion criteria

i. Any patient sent to the laboratory for brucellosis testing, based on clinical suspicion of having brucellosis by the medical officer at the study facility

ii. All consenting adults (18 years and above).

iii. Assenting minors aged 5-17 years with consent from their parents or guardian.

iv. Only those who have signed the informed consent forms were incorporated into the study.

Exclusion criteria

i. Human: Children less than 5 years of age.

ii. Anyone who didn't give informed consent or where assent was not granted.

3.5 Sampling Methodology

A risk-based sampling strategy was used, livestock populations found in areas with suspected or confirmed high incidence of brucellosis were targeted for identification of herds with previous symptoms of brucellosis. The practicing veterinary service providers, community leaders, livestock keepers and traders played a major role in identifying the herds, in addition to data generated from previous studies. The identified herds were visited for consenting from the household head. All the livestock species (cattle, sheep, goats, and camels) present at the household were considered for sampling. The household head, household members, together with the research team identified between 1 to 5 animals (from each species) with the highest likelihood of being *Brucella* positive, based on their history (as described in section 3.3-part b, and inclusion criteria 3.6.1).

3.5.1 Sample Size Determination

The formula for detecting presence or absence of a disease by Dohoo (Dohoo, 2010), below was used to estimate required sample size to be tested for detection of *Brucella*.

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

Where:

n = required sample size

α = 1-confidence level (usually = 0.05 for 95% confidence level)

D = estimated minimum number of diseased animals in the group (population size*minimum expected prevalence)

N= population size

To attain adequate sample size with a good precision (i.e. d=+/- 5 percent points), 95% level of confidence was used. The minimum numbers of diseased animals were estimated based on the livestock and human population (GoK_KNBS, 2017; KNBS, 2014, 2017), and the expected minimum prevalence of brucellosis within the study area. Expected prevalence of brucellosis for Narok and Northern Tanzania was assumed to be 15.3% for human, 3.3% for cattle, 3.6% for sheep and goats (small ruminants), based on the most recent study conducted in Kajiado County (Osoro et al., 2015). Kajiado County is occupied by the same Maasai community that are predominant in its neighboring county of Narok and the Northern part of Tanzania, where they also practice similar social cultural, and economic activities. Thus, the population is considered homogenous. The following prevalence values were used for Marsabit 46.5% for humans,11.2% cattle, 16.1% small ruminants and 11.1% for camels (Osoro et al., 2015). Calculation of wildlife samples was done using expected prevalence of 20.5%, which is an average of the prevalence values estimated for buffaloes (24%) and wildebeests (17%) in Ngorongoro area of Northern Tanzania (Fyumagwa et al., 2010), with estimated population of 1000 animals.

Proportions of the exposed animals and people were calculated to get (N) by multiplying the total population with expected prevalence. The minimum number of diseased animals (D) that reflect the different species of *Brucella* was estimated as (Nx0.05), assuming that each of the circulating species is present in at least 5% of the exposed population (N). The values (N, D and α) were substituted in the formula to get the expected number of positive samples needed from each host (n). The values for (n) were then adjusted to cater for potential clustering of *Brucella* species at the herd level using inter-cluster correlation coefficient (ICC) of 0.4 and an average sample size of 3 (1-5 animals) collected from each animal species at the herd or, this translated into a design effect of 1.8. to get the total number of samples needed for each host (Table 3.1).

Table 3.1: Variables used in calculating sample size, with sample values obtained with the formula

Site	Host	Total population	Prevalence (exposed) prop	Estimated exposed pop (N)	Min sub-group prevalence in N prop	Estimated min No. of each sub-group (D)	Alpha (α)	No. of unadjusted +ve animals (n)	Adjusted No. for ICC (0.4) and average No. of +ve animal species per herd (2.5) & design effect of 1.6
Marsabit	Cattle	218755	0.112	24501	0.05	1226	0.05	59	107
	goats	2029490	0.161	326748	0.05	16338	0.05	59	107
	Sheep	2029490	0.161	326748	0.05	16338	0.05	59	107
	Camel	217388	0.111	24130	0.05	1207	0.05	59	107
	Human	282472	0.465	131349	0.05	6568	0.05	59	107
Narok & Northern Tanzania	Cattle	1408198	0.033	46471	0.05	2324	0.05	59	107
	Goats	1117856	0.036	40243	0.05	2013	0.05	59	107
	Shoats	1117856	0.036	40243	0.05	2013	0.05	59	107
	Wildlife	10000	0.205	2050	0.05	103	0.05	58	105
	Human	393871	0.153	60262	0.05	3014	0.05	59	107
Total								**471**	**1068**

A total of 1068 animals and human samples were required, as summarized in Table 3.1 above.

3.6 Sample collection for identification of *Brucella* species in livestock and wildlife hosts

3.6.1 Livestock sampling

Livestock that meets the inclusion criteria, were enrolled into the study, and restrained appropriately with the help of the owner herder or neighbors. Vacutainer needle was used for collection of 10 ml of blood from the jugular vein into a plain vacutainer tube. Milk samples were collected directly from the animal's udder into a sterile 15ml falcon tube. Each sample tube

was labeled and kept upright for transportation to the laboratory using a cool box containing ice packs within 1 hour.

3.6.2 Wildlife Sampling

The sera samples from buffaloes were obtained from the Kenya Wildlife Services in Nairobi. These samples were opportunistically collected through routine veterinary interventions and disease surveillance activities conducted by the Kenya wildlife services between 2001 and 2021. Buffaloes were targeted due to several serological studies that have reported high prevalence of brucellosis in buffaloes as opposed to the other wildlife species (Ducrotoy et al., 2017; Fyumagwa et al., 2010). Majority of the samples (62) originated from Maasai Mara ecosystem (mainly Narok County and the surrounding counties), with 8 samples being sourced from Marsabit national park. The sera were transferred to the ILRI and kept at -20°C till tested.

3.7 Data Collection for Identification of *Brucella* Species in Humans

The medical facilities that serve people living within and around the sampled livestock populations were identified. Talek, Aitong, and Oloolaimutia dispensaries within Narok Counties were visited, for inclusion as study areas. The Marsabit County hospital, that functions as the referral hospital for the whole County as well as one dispensary in North Horr Sub-County were included for the Marsabit site. All the patients who were referred to the laboratory at each of the study facilities for brucellosis testing, due to clinical suspicion between September 2018 and April 2019, were eligible for the study. They were therefore, approached and included upon receipt of both oral and written informed consent.

3.7.1 Human Sample Collection

Laboratory technologists who are working at the selected medical facilities supported the sample and data collection. The venous blood was collected from study participants into a barcoded 6 ml plain vacutainer tube, upon obtaining oral and written informed consent or assent.

3.8 Data Collection for Assessment of Molecular Diversity and Transmission Dynamics of *Brucella* Species in different Hosts within Kenya and Tanzania.

3.8.1 Assessing Molecular Diversity and Transmission Dynamics

Information on *Brucella* species identified in animals and people (objectives 1 & 2) were merged and analyzed for variations in the proportions of *Brucella* species in the different animals and humans. A further analysis of the to establish the distribution of *Brucella* species in various

animal hosts and the different study areas was also performed. A model was then run to establish association between the different *Brucella* species and the different susceptible hosts.

3.8.2 Assessing Association between *Brucella* PCR Positivity and Exposure to the known Risk Factors for Transmission of Brucellosis

A semi-structured questionnaire was administered to each study participant by the clinician within the study facility to capture information on age, sex, and location of origin. Given that brucellosis in humans presents with a wide range of signs and symptoms that are similar to those presented by common tropical diseases such as malaria and typhoid (McDermott & Arimi, 2002; Muriuki et al., 1994), the clinical features were not investigated. Similarly, a semi-structured questionnaire was also administered at the household level to capture some of the known risk factors for transmission of brucellosis in animals for epidemiological analysis and transmission dynamics. Thus, the household head was interviewed to get information on the herd such as the animal species kept, history of abortion, history of retained placenta, age and sex of the animal sampled.

Association between *Brucella* PCR positive status and the risk factors for transmission was used to explain how transmission is occurring. This information was further used to explain the transmission dynamics of *Brucella* in the human-animal interphase.

3.9 Sample Labeling and Transportation

Each household was given a unique identification number, each livestock samples was then labelled with a barcode that also contained three digits that linked them to that of the household. Samples were kept upright in cool box containing ice packs before being transported to laboratory. On arrival in the laboratory, serum was obtained by centrifugation of blood in plain vacutainer tubes at 3000 rpm for 5-10 minutes and transferred into two cryovials with barcode labels. Then stored at -20°C together with the other set of samples until tested.

3.10 Laboratory Procedures

The lab procedure begun with DNA extraction from all the samples (serum and milk) by means of QIAamp DNA mini kit, (QIAGEN Germany). The extracted DNA were then tested for the presence of *Brucella* before identifying *Brucella* species from all the genus positive samples.

3.10.1 Preparation of the Milk Samples for DNA Extraction

Milk samples were brought to room temperature in a biosafety cabinet. After vortexing, 1ml of milk was aliquoted into 2ml Eppendorf tubes, and centrifuged at 8000rpm for 10 minutes. The top layers were decanted off using sterile methods leaving the pellets at the bottom of the tube for use as a primary material for DNA extraction.

3.10.2 DNA Extraction and Purification

DNA extraction is the first and crucial part of PCR based methods, the quality of DNA has an important contribution to the sensitivity of the PCR method (Smirnova et al., 2013). Genomic DNA was mined from serum and milk samples with the QIAamp DNA mini kit, (QIAGEN Germany). The kit was selected due to its ability to yield high quantity and quality DNA, a study that was conducted to evaluate the performance of commercial DNA extraction kits, ranked the QIAGEN kit as the best in giving high DNA yields, and reducing inhibition to improve results that are obtainable with the real-time PCR assays (Tomaso et al., 2010). The DNA extraction and purification process from both milk and serum samples were performed, based on the guidelines provided by the manufacturers. Briefly, 20 µl of proteinase K was added to 200 µl serum or pellet from each of the milk sample, 200 µl of lysis buffer added, then left for digestion at the room temperature for a period of 2 hours. This was followed by the transfer of the lysate into a spin column (provided with the kit), and manufacturers guidelines followed, before elution of the genomic DNA in 50 µl of elution buffer (Appendix 1). A NanoDrop 2000c Spectrophotometer (ThermoFisher Scientific, USA), was used to assess the purity and concentration of nucleic acid derived from each sample before being stored at -20°C till PCR was done. DNA purity was evaluated through an absorbent ratio of 260 nm / 230 nm and 260 nm / 280 nm, with values 1.8 to 2.0 and 1.7 to 2.4 consecutively being considered as "pure" (Sloan et al., 2021). The 260 nm / 280 nm values lower (≤1.6), indicated the presence of phenol, proteins, or any other contaminants that strongly absorb at or near 280 nm. While a lower value of 260 nm / 230 nm (≤1.6) indicated a potential extraction carryover.

3.10.3 Real-time PCR

The Real- time PCR was technique adopted for all the samples because it is a more rapid, more sensitive than conventional PCR, has a low limit of detection and it does not need post amplification of the PCR products for interpretation of results, hence lowering laboratory

contamination and false positives (Tomaso et al., 2010). However, given its high sensitivity, much effort was made to avoid any contamination of samples with amplicons in the laboratory. Preparation of PCR reaction mix, loading of samples and the actual running of PCR assays were performed in separated rooms, while using a different set of pipettes and lab coats for each of the three steps. In addition, negative controls (non-template) were used throughout the chain of preparation of DNA extraction, preparation of samples and PCR to check for any possible contamination. The PCR essays were first optimized to the local environmental conditions with reference to published standards (Appendix 2_PCR optimization standard curve). Each DNA sample was first tested in duplicate using primers targeting the Brucella-specific insertion sequence IS711 (Forward- GGC CTA CCG CTG CGA AT, Reverse- TTG CGG ACA GTC ACC ATA ATG; with a fluorescent probe, FAM- AAG CCA ACA CCC GGC-MGBNFQ) to detect the genus *Brucella* (Matero et al., 2011). A second *Brucella* genus detection assay targeting the Brucella-specific bcsp31 gene (Forward-GCTCGGTTGCCAATATCAATGC, Reverse- GGGTAAAGCGTCGCCAGAAG and a fluorescent probe 6FAM-AAATCTTCCACCTTGCCCTTGCCATCA-BHQ1) was also run on all samples as adapted from (Probert et al., 2004).

The size of the two PCR product were 178bP and 55bp for IS711 and bcsp31 primers, respectively (Bounaadja et al., 2009). An ABI 7500 thermocycler machine (Applied Biosystems, Life Technologies, Singapore) was used to perform PCR on all the samples. The PCR reaction mixture (20 µl) was prepared by mixing 0.5 µM of each of the primers with 4 µl of DNA template, , 10 µl of the qPCR master mix (Luna® Universal Probe 404 with UDG, from New England BioLabs, MA, USA) and 0.25 µM of fluorescent probe. The positive controls (*Brucella* controls comprising of, *B. abortus* 544 and *B. melitensis* 16M), that were utilized in the study were both obtained from the Friedrich-Loeffler-Institute *Brucella* Reference Laboratory in Germany). All the samples mixtures and non-template control were both tested in duplicates with the following PCR conditions; decontamination process at 50°C for 2 minutes, then polymerase activation and DNA denaturation at 95°C for 1 minutes, after that 40 cycles of 95°C for 15 seconds then 1 minute of 57°C. All samples that had amplification and a cycle threshold (ct) value <40 for both genus targets in one, or both of the duplicate wells were considered as *Brucella* genus PCR positive. Samples positive on this step were later taken through a multiplex PCR speciation assay (Probert et al., 2004) with oligonucleotide primers and probes detecting

specific IS711 insertions downstream of the alkB gene for identification of *B. abortus* (Forward-GCGGCTTTTCTATCACGGTATTC, Reverse- CATGCGCTATGATCTGGTTACG and a fluorescent probe: JOE-CGCTCATGCTCGCCAGACTTCAATG-BHQ1) and downstream of the BMEI1162 locus for *B. melitensis* (Forward-AACAAGCGGCACCCCTAAAA, Reverse CATGCGCTATGATCTGGTTACG, and a probe, Texas Red-CAGGAGTGTTTCGGCTCAGAATAATCCACA-BHQ2). Samples were run under identical reaction conditions on an ABI 7500 thermocycler. Any sample with amplification in the respective target and value of a cycle threshold (ct) that is less than 40 was interpreted as positive for either *B. melitensis* or *B. abortus*. A run was only considered valid if all negative/ no-template controls did not amplify, and positive controls amplified within agreeable range of the standard curve equivalent. A test run was classified as valid when amplification was observed for positive controls and no amplification was observed for the negative controls.

3.11 Data Analysis

Data was entered into MS Excel version 2018 (IBM, California), where they were cleaned and merged. The R statistical software version 3.6.3 (R Core Team, 2008) was then utilized to perform all the data analysis. Descriptive statistics was performed using the combined data set from all the sites to establish proportions of PCR positivity with variables like age, sex, location of origin, animal species, history of abortion or retained placenta. Further analysis for objectives one and two were performed using combined data of *B. melitensis* and *B. abortus* outcomes data from livestock, and human were to evaluate the influence of host species on *Brucella* species detection. The percentage positive for each test by species and site was calculated, with binomial exact confidence intervals using *gmodels* and *binom* functions in R statistical software.

Mixed effects logistic regression models created with the function *glmer* in the package *lme4* (Bates et al. 2014) with sampling location included as the random effect, and the data being specified as having binomial distribution, were performed to assess association between *Brucella* spp. PCR test status in livestock and human populations for the third objective. Variables that were evaluated for association with PCR status in the human model included gender, and age category. For livestock, history of abortion, history of retained placenta and animal spp. variables were incorporated in the model as possible predictors for *Brucella* PCR status. In this model, camels and male ruminants were not included because of the low numbers of observations and

the absence of appropriate clinical history (retained placenta and abortion) of the assessed variables, respectively. Pigs and buffaloes were also excluded from all the models due to missing data on the assessed variables.

Both models were fitted with sampling location included as a random effect, and the data being specified as having binomial distribution.

The maximal models were simplified by considering a p-value less than or equal to 0.05 as having statistical significance to get the final models. Intra-cluster correlation coefficients (*icc*) for within-location clustering of brucellosis for both livestock and humans were calculated from the variance components of the final multivariable models using an *icc* function contained within *sjstats* package (Brecht et al., 2015).

3.12 Ethical Considerations

The study proposal and protocols were reviewed and approved by the National Commission for Science, Technology, and Innovation (Ref. no. NACOSTI/P/19/81438/29438) (Appendix 3). Additional approvals for the livestock components was also granted by the Institutional Animal Care and Use Committee at the international Livestock Research Institute (Ref. no. ILRI-IACUC2018-16) (Appendix 4), while the same institution also awarded an approval for the human component upon being reviewed by the ILRI's Research Ethics Committee (Ref. no. ILRI-IREC2018-14) (Appendix 5). All the relevant guidelines and regulations recommended by the above research-governing committees were strictly followed during the study implementation period. Import permit was obtained before bringing samples from Tanzania (Appendix 6). Copies of approval forms, research proposal abstract, as well as University approval (Appendix 7) were submitted to the County Directors of Livestock and as well as Public Health for Narok and Marsabit for update, guidance and provision of permission and support before implementing the study. Therefore, oral, and written approvals were received from the counties before collection of data (Appendix 8). Both oral and written informed consent were sought and obtained from all adult participants (18 years and above), whereas parent's consent was received for participants below 12 years of age (Appendix 9). Consent and assent were also obtained from parent or guardian before enrolling persons between 13–17 years of age into the study. The household head provided an informed consent prior to the inclusion of a herd of individual animals in the study. The research proposal together with informed consent forms and detailed

protocols were also reviewed and approval by the ethical review committees, based at the National Institute of Medical Research (NIMR), Tanzania (NIMR/HQ/R.8c/Vol. I/1140), (NIMR/HQ/R.8a/Vol. IX/3102) (Appendix 10).

The ethical consideration included; confidentiality, good justification of sample size, and analyses with anonymous data sets; minimization of potential risks by application of best practices and professional handling, safe storage of questionnaires data, interviews being conducted in private environments and commitment to share key research findings with the study participants.

3.13 Validity and reliability of research findings

The field protocols were pretested while laboratory protocols optimized to ensure that the research findings are reliable. The following activities were performed.

i. A total of ten herds were selected and sampled in both Marsabit and Narok counties for pretesting of the research protocols, such as the consenting process, inclusion and exclusion criteria, questionnaires, sample collection and management process. The data generated from pretesting were not included into the study.

ii. The study adopted previously validated and published primers, probes, and protocols.

iii. PCR assays were optimized using positive controls from a *Brucella* reference lab (Friedrich-Loeffler-Institute *Brucella* Reference Laboratory in Germany). Furthermore, a standard curve assay was also run to ensure that the Ct values were within the expected range.

iv. Only runs that had no amplifications on all negative/no-template controls, and clear amplifications of positive controls within agreeable range of the standard curve equivalent were considered as valid.

v. Two assays with different targets were run independently to detect the genus *Brucella* DNA from each sample, with each sample being tested in duplicates.

CHAPTER FOUR

RESULTS

4.1 Identification of *Brucella* animals

4.1.1 Summary of Animal Population and *Brucella* spp. PCR Results

The demographic features of the sampled livestock are summarized in Table 4.1. A total of 1384 livestock including 709 cattle, 274 goats, 191 sheep, 79 pigs, 70 buffaloes, and 61 camels were sampled in both Kenya and Tanzania. Camels were only sampled in Marsabit County as these animals were not kept in sampled regions in Narok and Northern Tanzania. The overall PCR positivity of *Brucella* spp. in livestock was 22.0% (95%, 19.9-24.3). PCR positivity of *Brucella* spp. differed significantly between livestock species, with the highest being recorded in camels (59.0%, CI 45.7-71.4), followed by buffaloes (30.0%, CI 22.0-39.0), sheep (37.6%, CI 30.3-45.4), goats (26.9%, CI 21.4-33.0), pigs (21.5%, CI 13.9-31.1), and cattle (17.7%, CI 13.6-22.6). A higher proportion of male animals (41.7%) tested positive compared to female animals (28%). A higher proportion of positive cases was found in livestock (38.8%) from Narok, compared to (23.5%) from Marsabit, and (12.7%) Tanzania.

Table 4.1: Summary of livestock (cattle, goats, sheep, and camel) population composition, descriptive characteristics, and *Brucella spp*. PCR results

Variables		**Northern Tanzania**		**Narok**		**Marsabit**		**Combined data**		
Variable	Category	Total	No +ves	Total	No +ves	Total	No +ves	Total	Total +ves	% PCR positive (95% CI)
Sex	Male	19	0	21	7	27	13	67	20	29.9 (19.3 – 42.3)
	Female	492	58	234	92	442	97	1168	247	21.1 (18.8 – 23.6)
Abortion	No	442	45	230	86	408	88	1080	219	20.2 (17.9 – 22.6)
	Yes	30	13	25	13	61	22	116	48	41.4 (32.8 – 50.8)
Retained placenta	No	500	57	250	96	458	106	968	9	0.9 (0.4 –1.8)
	Yes	1	1	5	3	11	4	267	8	3.0 (1.3 – 5.8)
Species	Cattle	451	53	55	24	203	19	709	96	13.5 (11.1 –16.3)
	Goats	30	4	78	35	166	31	274	70	25.5 (20.5 – 31.1)
	Sheep	20	1	122	40	49	24	191	65	34.0 (27.3 – 41.2
	Camels	-	-	-	-	61	36	61	36	59.0 (45.7 – 71.4)
	Pigs*	59	13	20	4	-	-	79	17	21.5(13.9 – 31.1)
	Buffaloes			62	17	8	2	70	19	30.0 (22.0 – 39.0)
Herd comp	single	501	58	58	22	101	41	669	120	17.9 (15.1 – 21.1)
	mixed	0	0	197	77	368	69	566	147	26.0 (22.4 – 29.8)
Total	Total samples	560	71	295	118	479	110	1384	303	22.0 (19.9 – 24.3)

Key: CI Confidence Interval, PCR Polymerase Chain Reaction, No +ves number of positive sam ples, Total +ves total number of positive samples. Pigs* pig samples were collected in areas outside the proposed study sites.

4.1.2 Proportion of *Brucella* Species Detected in Animals

Of 303 *Brucella* spp. PCR positive animal samples, 120 (39.6%) were identified as *B. abortus* and 113 (37.3%) as *B. melitensis*, while 80 (26.4%) of the genus PCR positive samples did not amplify with either *B. abortus* or *B. melitensis* primer targets (Table 4.2). *Brucella abortus* was detected in camels (61.1%), cattle (41.7%), goats (25.7%), sheep (20.0%), pigs (42.1%) and buffaloes (52.7%), while *B. melitensis* was found in sheep (63.1%), goats (48.6%), camel (22.2%), cattle (29.2%) and buffaloes (11.8%). A big proportion of pig samples (57.9%) did not amplify with both *B. abortus* and *B. melitensis* targets (Table 4.2).

Table 4.2: Proportions of *B. melitensis* and *B. abortus* detected from different livestock hosts (cattle, sheep, goats, camels, buffaloes, and pigs) based on univariable analysis

Variables		**Total number of *B. abortus* detected**		**Total number of *B. melitensis* detected**		**Undetermined**	
Livestock host	**Total**	**No. positives**	**% positivity and (95%CI)**	**No. positives**	**% positivity and (95%CI)**	**No. positives**	**% positivity and (95%CI)**
Cattle	96	40	41.7 (31.6-52.8)	28	29.2(20.3-39.3)	28	29.2 (20.3-39.3)
Sheep	65	13	20.0 (11.1-31.8)	41	63.1 (50.2-74.7)	11	16.9 (8.8-28.3
Goats	70	18	25.7 (16.0-37.6)	34	48.6 (36.4-60.8	18	25.7 (16.0-37.6)
Camel	36	22	61.1% (43.5-76.9)	8	22.2 (10.1-39.2	6	16.7 (6.4-32.8)
Pigs	19	8	42.1 (20.3-66.5)	0	0.0 (0-17.6)	11	57.9 (33.5-79.7)
Buffaloes	17	9	52.9 (27.8-77.0)	2	11.8 (1.5-36.4)	6	35.3 (14.2-61.7)
Total	303	120	39.6 (34.1-45.4)	113	37.3 (31.8-43.0)	80	26.4 (21.5-31.7)

4.2 Identification of *Brucella* Species in Humans

4.2.1 Humans Demographics

A total of 247 humans (110 in Narok and 147 in Marsabit) were sampled. Most samples were collected from female participants (n=148) and the participants' age varied from 3 to 96 with a mean age of 32.6 years. Individual age was recorded as continuous variable, but since it did not meet the linearity postulation, it was categorized into 3 levels (<20 years, between 20 and 40 years and those above 40 years). The total positive samples for female participants was 59/148 compared to 40/109 in male participants. The overall PCR positivity of *Brucella* spp. in humans

was 40.1% (95% CI 32.5-44.7). A higher proportion of positive cases was found in humans (40.8%) from Narok, compared to humans (35.5%) from Marsabit. The number of seropositive samples detected in humans was 46 (19.3%; 95%CI 14.5-24.9), and only 43.5% (95%CI 28.9-58.9) of the seropositive samples tested positive with *Brucella* genus PCR test (Table 4.3).

Table 4.3: Risk factors associated with *Brucella spp.* PCR positive cases in humans, based on Univariable analysis

		Narok		**Marsabit**		**Combined human data**		
Variable	Category	Total (N)	No. positive	Total (N)	No. positive	Total (N)	No positive	% PCR positive (95% CI)
Gender	Male	55	22	54	18	109	40	36.7 (27.7-46.4)
	Female	92	38	56	21	148	59	39.9 (31.9-48.2)
Age category (years)	≤ 20	39	13	18	2	58	16	27.6 (16.7-40.9)
	21-40	65	36	54	24	99	52	52.5 (42.2-62.7)
	> 40	23	8	10	3	54	17	31.5 (19.5-45.6)
Serology test	Negative	116	31	76	25	192	58	30.2 (26.2-34.4)
	Positive	48	12	15	7	46	20	43.5 (28.9-58.9)
Site	Marsabit	147	60	110	39	257	99	38.5 (32.5-44.8)

4.2.2 Proportion of *Brucella* Species Detected in Humans

A total of 43 samples were found to be positive for *B. abortus*, while 29 sampled positive for *B. melitensis* (Table 4.4).

Table 4.4: showing the proportion of *B. melitensis* and *B. abortus* identified in humans

Brucella species	**Total tested**	**Total detected**	**% positivity and 95% CI**
B. abortus	99	43	43.4 (34.3-54.0)
B. melitensis	99	29	29.3 (21.2-38.8)
Undetermined *Brucella* spp. results		27	27.3 (19.2-36.3)

4.3 Molecular Diversity and Transmission Dynamics of *Brucella* Species in Different Hosts within Kenya and Tanzania

Results from several sub-topics were analysed to understand the molecular diversity and transmission dynamics of *Brucella* spp. The investigated sub-topics included.

i. Distribution of *B. melitensis* and *B. abortus* within the different study areas.

ii. Association between *B. abortus* and *B. melitensis* and targeted hosts.

iii. Factors associated with *Brucella* spp. PCR positive cases in livestock.

iv. Factors associated with *B. melitensis* and *B. abortus* in livestock.

v. Factors associated with *Brucella* spp. PCR status in humans.

4.3.1 Distribution of *B. melitensis* and *B. abortus* within the different Study Areas of Narok, Marsabit in Kenya and Northern Tanzania.

A relatively higher proportion of *B. abortus* was detected in Marsabit 48.3% (95% CI: 40.0-56.6) as opposed to Northern Tanzania 31.0 (95% CI: 19.5-44.5) and Narok 29.6% (95% CI: 22.6-37.3). While higher proportion of *B. melitensis* were observed in Narok 45.9% (95%CI: 38.0-54.0), followed by Northern Tanzania 37% (25.5-51.6), then Marsabit 30.2% (95% CI:23.0-38.3) (Figure 4.1).

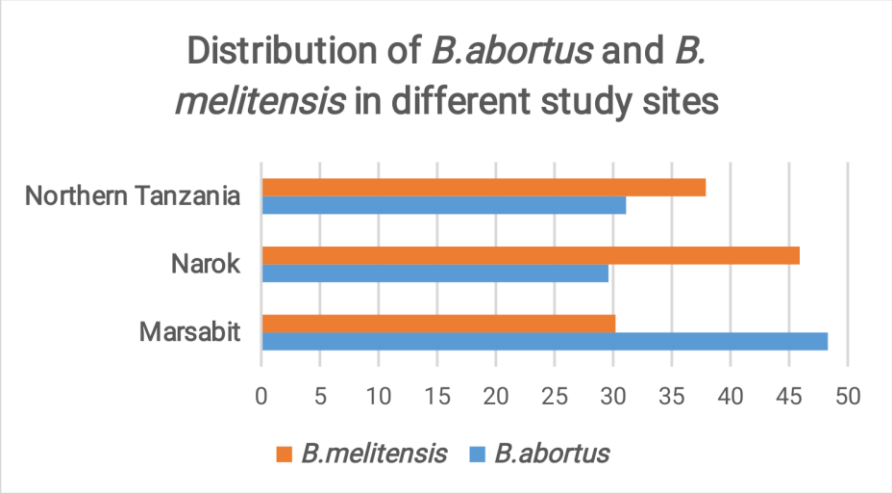

Figure 4.1: Distribution of *B. melitensis* and *B. abortus* in Narok, Marsabit in Kenya and Northern Tanzania

The chi-square test was performed to assess the distribution of *B. abortus* within the three sites and the results established a significant variation in the distribution of *B. abortus* in the three study sites (p=0.002). Similarly, significant variation was detected in the distribution of *B. melitensis* in the three study sites (p= 0.018).

4.3.2 Association between *B. melitensis* and *B. abortus* and the Targeted Hosts

The results obtained from multivariable mixed-effect models run on cattle, sheep, goats, camels, and human data found a significant absence of association between *B. abortus* was significantly associated with sheep and goats while there was no significant evidence of lack of association between *B. melitesis* with cattle and camels (Table 4.4).

Table 4.5: Association between *B. melitensis* and *B. abortus* with the targeted hosts (cattle, sheep, camels, and goats) based on univariable and multivariable mixed-effects logistic regression models with random effects for sampling (degrees of freedom=3, number of observations=209)

Outcome	**Level**		
	Host	Odds Ratio 95% CI	Odds ratio *P*-value
B. abortus	Cattle (baseline)	1	
	Goats	0.5 (0.2 - 1.1)	0.071
	Sheep	0.4 (0.2 - 0.8)	0.017
	Camel	1.6 (0.6 - 4.1)	0.332
	Human	0.8 (0.4 - 1.7)	0.595
B. melitensis	Goats (baseline)	1	
	Cattle	0.5 (0.2 - 1.0)	0.043
	Sheep	1.9 (0.9 - 3.9)	0.092
	Camel	0.4 (0.2 - 1.2)	0.113
	Human	0.6 (0.3 - 1.3)	0.181

4.3.3 Factors associated with *Brucella* spp. PCR positive cases in livestock

There was a significant positive relationship between animals that had history of abortion and *Brucella* PCR positive status. Animals with history of abortion were also found to have elevated odds (OR=2.0, 95% CI 1.1-3.5) of testing positive by PCR. Keeping animals in mixed herds (OR=0.6, 95% CI 4.0-0.9) and the type of animal species kept (OR=1.9, 95% CI 1.6-2.3) were associated with *Brucella* PCR positivity (Table 4.5). The *icc* for within-location clustering of *Brucella* PCR positive status for the livestock model was estimated to be 0.4.

Table 4.6: Summary of multivariate mixed-effects logistic regression models run to identify variables associated with *Brucella spp.* PCR status in livestock

	Variables	*Odds ratio (95% CI)*	***Odds ratio*** *P-value*
PCR positivity	Abortion	2.0 (1.1-3.5)	0.022
	Mixed herd composition	0.6 (0.4-0.9)	0.01
	Animal species	1.9 (1.6-2.3)	<0.001

Location *icc* = 0.4

Key: CI Confidence Interval, P-value according to Pearson Chi-Square test, *icc* intra-cluster correlation coefficients.

4.3.4 Factors Associated with *B. melitensis* and *B. abortus* in Livestock

Results from multivariable mixed effect logistic regression models found a positive association between *B. abortus* and mixed herds (OR=0.6, 95% CI 0.4-0.9), likewise, animals with history of abortion (OR=2.0, 95% CI 1.1-3.5), and the association between *B. abortus* independent animal species, varied significantly (OR=1.9, 95% CI 1.6-2.3). *B. melitensis* was also associated with mixed herd (OR=0.5, 95% CI 0.3-0.8), retained placenta (OR=3.1, 95% CI 2.6-3.8), and animal species (OR=1.9, 95% CI 1.0-3.3) (Table 4.6).

Table 4.7: Variables associated with *B. melitensis* and *B. abortus* PCR positivity in livestock using the multivariate mixed-effects logistic regression models

Variables	Variables	Odds ratio (95% CI)	Odds ratio *P*-value
B. abortus	Animal spp.	1.9 (1.6-2.3)	0.000
	Mixed herd	0.6 (0.4-0.9)	0.010
	abortion	2.0 (1.1-3.5)	0.022
B. melitensis	Animal spp.	1.9 (1.0-3.3)	0.000
	Mixed herd	0.5 (0.3-0.8)	0.008
	Retained placenta	3.1 (2.6-3.8)	0.037

Key: CI Confidence Interval and P-value according to Pearson Chi-Square test.

4.3.5 Factors associated with Brucella spp. PCR status in humans.

The final model fitted for human *Brucella* spp. PCR status only had age category as a significant variable positively associated with *Brucella* PCR status (Table 4.7). Individuals between21 and 40 years of age were more likely to be PCR positive than individuals with an age category of less or equal to 20 years (Table 4.8). Sex was not significantly associated with PCR status; therefore, was dropped from the final model. The *icc* for within-location clustering of human brucellosis estimated for this model was < 0.001.

Table 4.8: Summary of the final mixed-effects logistic regression models run to assess associations between variables and *Brucella spp.* PCR status in human

Variables	Category (years)	Odds ratio (95% CI)	Odds ratio *P*-value
Age category	≤ 20	1 (baseline)	
	21-40	2.8 (1.2-6.6)	0.016
	> 40	1.2 (0.4-3.1)	0.771

Key: CI Confidence Interval, P-value according to Pearson Chi-Square test.

CHAPTER FIVE

DISCUSSION

5.1 Detection of *Brucella* Species Circulating in Animal Population

This study detected *B. melitensis* and *B. abortus* and in goats and sheep. Both *B. melitensis* and *B. abortus* have been reported in goats in the East African region (Mahlau, 1967; Ntirandekura et al., 2020; Philpott & Auko, 1972; Wainaina et al., 2020). However, information on species of *Brucella* species circulating in sheep has been missing. Therefore, the findings from this current study has generated new information on circulating species of *Brucella* in the sheep populations in East Africa. Detection of *B. melitensis* and also *abortus* in sheep and goats has been observed in other regions of the world (Ashrafganjooyi et al., 2017; Caine et al., 2016; Currò et al., 2012; Hamdy & Amin, 2002).

Results from this study also found that *B. melitensis* plus *B. abortus* to be circulating in cattle population. Many studies have reported similar findings within East Africa (Mahlau, 1967; Mathew et al., 2015; Muendo et al., 2012; Mugizi et al., 2015) and elsewhere (Dadar et al., 2019; Hamdy & Amin, 2002; Selim et al., 2019). This is the first study to ever report the presence of *B. abortus* in camels within the East African region. *Brucella melitensis* was also found in camels, which is comparable with findings from a recent study in Kenya (Muturi et al., 2021). The findings from this study agrees with earlier reports that camels are susceptible to *B. melitensis* and *B. abortus* (Fatima et al., 2016; Gwida et al., 2012; Hamdy & Amin, 2002; Kaltungo et al., 2014). The findings from the study further confirmed the circulation of *B. abortus* and *B. melitensis* in buffaloes, which is similar to previous reports from Sri Lanka and Iran (Dehkordi et al., 2014; Priyantha, 2011).

This is also the first study to report *Brucella* spp. in circulating in the pigs within the in East African region using molecular techniques. Presence of the zoonotic *Brucella* spp. in the pig sera, and especially, *B. abortus*, is significant due to the fact that pigs are by tradition known to have an association with *B. suis*, and not *B. abortus* that is recognized to have cattle as their preferential host. Therefore, this finding underlines the possible existence of a unique transmission dynamic in pigs that require additional studies to inform appropriate control options for brucellosis control in Kenya, Tanzania, and other similar settings.

5.2 Identification of *Brucella* spp. Circulating in the Human Population

This study detected *B. melitensis* and *B. abortus* in humans in Narok and Marsabit. This finding is in harmony with results from previous investigations in East African region (Bodenham et al., 2020; Muendo et al., 2012; Oomen, 1976) and elsewhere (Dadar et al., 2019; Lucero et al., 2008). Other *Brucella* species including *B. suis* and *B. canis* and *B. canis* have also been reported in humans (Lucero et al., 2008; Pappas, 2010; Pappas et al., 2006; Suárez-esquivel et al., 2017). Although, only *B. melitensis* and *B. abortus* were observed to be circulating in the human population in the study region, the presence of other *Brucella* species in this population cannot be ruled out given that 26.3% of PCR positive samples did not amplify with *B. melitensis* and *B. abortus* targets, used in the study.

Multiple animal species could be responsible for transmitting *Brucella* spp. to humans in Kenya and Tanzania, since both *B. abortus* and *B. melitensis* detected in humans were also found in cattle, goats, sheep, and camels. Therefore, all the livestock species should be targeted in brucellosis control programs, such as vaccination of animals, and public health education that are aimed at reducing *Brucella* infection in humans.

5.3 Molecular Diversity and Transmission Dynamics of *Brucella* species in Humans and the Different Animal Hosts within Kenya and Tanzania

Detection of *Brucella* in all the animal species investigated in this study is an indication of a complex epidemiology. This study found that camels had the highest proportion of *Brucella* PCR positivity in comparison to other animal species. This could be ascribed to the fact that camels are mostly kept in arid areas that are characterized by high rate of migration (O'Connor et al., 2016) that leads to increased rate of sharing of watering points and grazing areas or direct interaction of different herds, thereby enhancing their exposure to *Brucella* spp. and other infectious pathogens. The presence of zoonotic *Brucella* species in camels could present public health challenge, given the steady rise in camel populations in Kenya (FAO, 2002), primarily for milk production (Masinga et al., 2008; Watson et al., 2016). Consumption of raw milk (Osoro et al., 2015), and the significant increase in production and extensive distribution of camel milk (Masinga et al., 2008; Noor et al., 2012), further strengthen the need the for designing more studies and intervention programs that are aimed at mitigating the potential role of camels in zoonotic transmission of *Brucella*, and other potential pathogens.

People within the age category of 21-40 years had an elevated likelihood of getting exposed to *Brucella* PCR positive status. Earlier studies have also reported higher prevalence rate in humans within the similar age category (Alkahtani et al., 2020; Muloki et al., 2018). Given the observed role played by those within the age category of 21-40 years during the field data collection. The high positivity rate could be attributed to their prime responsibility of milking, helping animals during parturition and herding. Hence, making them more exposed as opposed to the younger population (below 21 years) that are mostly school going or have a relatively lower contact when taking care of animals. Persons above 40 years of age tend to take more leadership roles, while reducing their active participation in taking care of animals, thus lowering their risk of getting exposed to zoonotic pathogens due to regular contact with the livestock or the contaminated animal products. This result is comparable to earlier studies (Assafi & Al-berfkani, 2019) that reported an elevated prevalence of brucellosis in the same age category and associated this to their occupational roles with livestock. The distribution of *Brucella* positive cases across all the age groups suggests that consumption of contaminated animal products could be an alternative transmission route as has been reported in different studies in the region (Bodenham et al., 2020; Migisha et al., 2018). Previous studies had recommended the use of real-time PCR assays as rapid and sensitive tests for the detection of *Brucella* spp. (Al Dahouk et al., 2007; Matero et al., 2011). This study utilized DNA samples extracted from serum and milk to test for *Brucella* positivity. Earlier studies had demonstrated the suitability of using serum as a suitable sample type for extraction of DNA for the detection of *Brucella* in humans and livestock (Zerva et al., 2001). Utilization of same sample type for both direct nucleic acid detection and serological testing of *Brucella* is an emerging field diagnosis for brucellosis (Dahouk et al., 2004; Zerva et al., 2001). Additional advantage of bypassing the dangerous and tedious culture procedures makes PCR a powerful and convenient brucellosis surveillance tool. Future studies should consider generation of more data to explore the agreement between molecular approaches and serology, and how each approach informs the infection status in humans and animals.

In this study, location was used as random effect to take cater of the possible clustering effects of *Brucella* PCR positive cases in the mixed effect models. An *icc* 0f 0.4 was estimated for location using the livestock dataset. This suggests that brucellosis in livestock often cluster within locations, frequent or close interaction of herds kept within the same location could be a major contributor to the clustering of *Brucella* cases within a location. However, there was no

clustering of human cases by locations (*icc*=0). This lack of clustering of *Brucella* in humans may be attributed to the fact that humans may get exposed to brucellosis in other locations other than their normal residential locations through consumption or due to contact with infected or else contaminated animal products. The wide distribution of dairy products, especially milk in Kenya (Kiambi et al. 2018) may also be attributed to lack of clustering of brucellosis in humans.

Brucella melitensis and *B. abortus* were detected in wildlife as well as various livestock species. Detection of both *Brucella* species across all hosts is consistent with previous reports that *Brucella* spp. are not entirely host specific, but cross transmission of *Brucella* spp. between different susceptible hosts is possible in areas where different animal species interact within a close range (Godfroid et al., 2013). This finding further supports earlier serological indication of a possible cross transmission of *Brucella* species between livestock and wildlife (Enström et al., 2017; Gomo et al., 2012), given the presence of same species of *Brucella* in both hosts. Keeping of animals in mixed herds, using same grazing areas, in addition to congregation of animals around the communal watering sites have been recognized as factors that facilitate the transmission of *Brucella* species (Kairu-Wanyoike et al., 2019). Therefore, this could have contributed to the observed cross transmission of *Brucella* spp. within the two pastoral areas studied. *Brucella abortus* was found to have a significant association with cattle and camels, whereas *B. melitensis* was the main *Brucella* spp. associated with infection in goats and sheep. This finding is similar with existing knowledge on preferential nature of *Brucella* spp. in different animal species (OIE, 2016).

Animals with history of abortion were significantly associated with *B. abortus* PCR positive status. This suggest that contamination of grazing areas or direct contact with aborted materials from *Brucella* infected animal could be one of the leading modes of transmission of *B. abortus* between different animals as well as humans. Occurrence of retained placenta in animals were strongly associated with *B. melitensis* cases, indicating that transmission of *B. melitensis* through direct contact with placenta or contaminated surfaces with placenta from animals with *Brucella* infection could be one of the major routes of transmission for *B. melitensis*. Previous studies have reported that large numbers of *Brucella* organisms are predilates within placenta and aborted fetuses from *Brucella* infected animals (Samartino & Enright, 1993). Therefore, highlighted the important role that aborted materials and retained placenta could be playing in

facilitating the transmission of *Brucella* within livestock and wildlife populations, as well as cross transmission between livestock, wildlife, and humans, especially in areas where close interaction is common.

5.4 Study Limitations

This study had the following limitations. First, the targeted study design may have led to the relatively higher PCR positivity reported in this study, although it also limits the power of making inference to population level prevalence. Secondly, only *B. abortus* and *melitensis* were targeted by the PCR speciation assay used in this study. Thus, 22.8% of the genus *Brucella* PCR positive samples that had no amplification with both targets could not be identified, and the potential circulation of other *Brucella* species within the targeted population could have been missed. More epidemiologically focused studies should be done using real-time PCR with more typing options to address the prevailing challenges in the understanding of molecular epidemiology of *Brucella* in the region.

CHAPTER SIX
SUMMARY OF RESEARCH FINDINGS, RECOMMENDATIONS, AND CONCLUSIONS

6.1 Summary of Research Findings

This is among the very first studies in the region to undertake a population level molecular study aimed at detecting circulating species of *Brucella* in several livestock hosts and humans. This study provided more proof of the existence of *B. melitensis and B. abortus* in several animal species in Kenya. Both *B. abortus* and *B. melitensis* were detected in sheep, cattle, goats, camels, buffaloes, besides humans. This is also the first study to detect *B. abortus* in pigs in Africa, and to also use molecular technique to demonstrate occurrence of clustering of *Brucella* cases in different geographical locations.

Humans were found to be exposed to *B. melitensis* in addition to *B. abortus*, suggesting that cattle, sheep, goats, pigs, camels, besides buffaloes might be contributing to zoonotic transmission of *Brucella* spp. The study further highlighted associations between the different susceptible hosts *Brucella* species. Furthermore, an indication potential occurrence of cross transmission of *Brucella* species in areas with close interactions between the various animal species. Finally, occurrence of retained placenta and or abortion were identified as significant predictors for PCR positivity because of *B. melitensis* and *B. abortus* in livestock, respectively. People within age category of 21 to 40 years were associated with *Brucella* PCR positive status, partly due to their high level of contact during herding, milking, and helping animals during parturition.

6.2 Conclusion

i. Two *Brucella* species *B. melitensis* and *B. abortus* were identified as major circulating in different animal hosts. Both species were found to be circulating in sheep, goats, cattle, and camels. Only *B. abortus* was found to be circulating in the pig populations.

ii. Both *B. melitensis* and *B. abortus* were found to be the major contributors to human infections in Marsabit and Narok, Kenya.

iii. The findings confirmed that *B. melitensis* have a positive and significant association with goats, and sheep, while *B. abortus* is associated with cattle, pig, and camels. *Brucella* PCR status is influenced by the history of retained placenta and abortions in animals,

while persons within the age category with the highest contact with animals were found to have the highest exposure rate.

6.3 Recommendations from this Study

i. Brucellosis control in animals using strategies such as vaccinations should target all livestock hosts.
ii. Multiple livestock species may be responsible for transmitting *Brucella* to humans. Therefore, control programs in Kenya should use a *One Health* strategy targeting multiple host species including cattle, sheep, goats, pigs, and camels to effectively control brucellosis in humans.
iii. Proper disposal and handling of aborted materials and retained placental tissues should be considered as one of the ways of reducing the risk of transmitting *Brucella* spp. Contact between different animal species should also be reduced to avoid cross transmission of different Brucella species.

6.4. Recommendations for Future Work

1. More targeted studies should be developed to systematically characterize the strains and biovars of *Brucella* in pigs and wildlife species.
2. Expanding the range of real-time PCR typing options should be considered in future studies to shed more light on all the *Brucella* species present in the targeted population.
3. A longitudinal study should be conducted at the wildlife and livestock interfaces to establish the direction of spread of *Brucella* species between livestock and wildlife.

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APPENDICES

Appendix 1: DNA Extraction Protocol

1. Pippete 20 µl Qiagen proteinase K into the bottom of 1.5ml microcentrifuge tube.

2. Add 200 µl sample to the microcentrifuge tube containing 20 µl of proteinase K

3. Add 200 µl buffer AL

4. Incubate at 56°C for 1hour.

5. Briefly centrifuge the 15ml microcentrifuge tube to remove the drops from the inside of the lid

6. Add 200 µl ethanol (96 – 100%) to the sample, then mix by pulse vortexing for 15 seconds. After mixing, briefly centrifuge to remove drops from inside.

7. Carefully apply the mixture from step six to the QIamp mini spin column 2ml collection tube without wetting the rim. Close the cap and let it stand for 30 minutes before centrifuging at 8000rpm for 1 minute.

8. Place the Qiamp minim spin column in a clean 2ml collection tube and add 500 µl of buffer AW1 without wetting the rim. Close and centrifuge at 8000rpm for 1 minute. Then place the spin column in another clean 2ml collection tube.

9. Carefully open the spin column and add 500 µl of buffer AW2 and spin at full speed of 14000rpm for 3minute, then transfer the spin column to another clean 2ml collection tube for a further centrifugation at 14000rpm for 1 minute.

10. Place the Qiamp mini spin column in a clean 1.5 microcentrifuge tube. Carefully open

Appendix 2: Standard curve with Brucella positive controls, showing efficiency of the PCR assay

Positive controls received

1 µg vacuum dried purified DNA of:

Brucella species	**Biotype**	**Strain**
Brucella abortus	Biotype 1	Reference strain 544
Brucella melitensis	Biotype 1	Reference strain 16M

1. **Reconstitution and Dilution**

Reconstitution of the controls stock DNA was done by adding 100 µl of RNase-DNase free water to the freeze-dried samples. The final concentration of stock DNA was 10ng/µl.
10-fold serial dilutions were prepared for the working concentration and for generation of a standard curve at qPCR.

2. **qPCR Results**

The multiplex reaction worked as expected. No contamination was observed in the negative controls. All the targets (genus *Brucella*, *B. melitensis*, and *B. abortus*) had amplifications as below;

<u>Standard curve for the genus *Brucella* target (Bcsp31)</u>

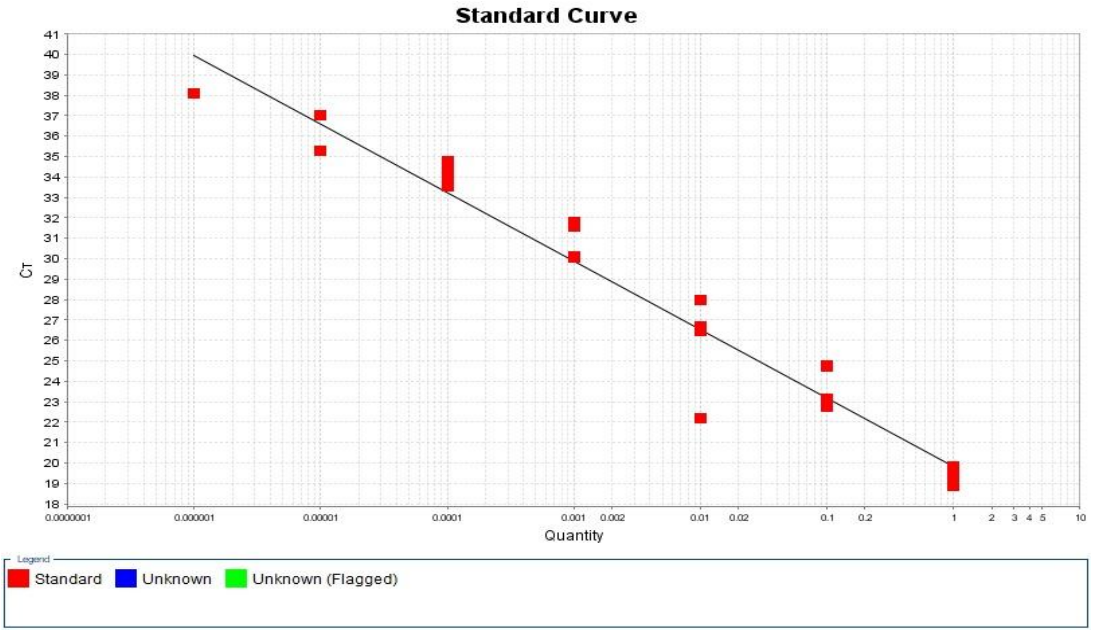
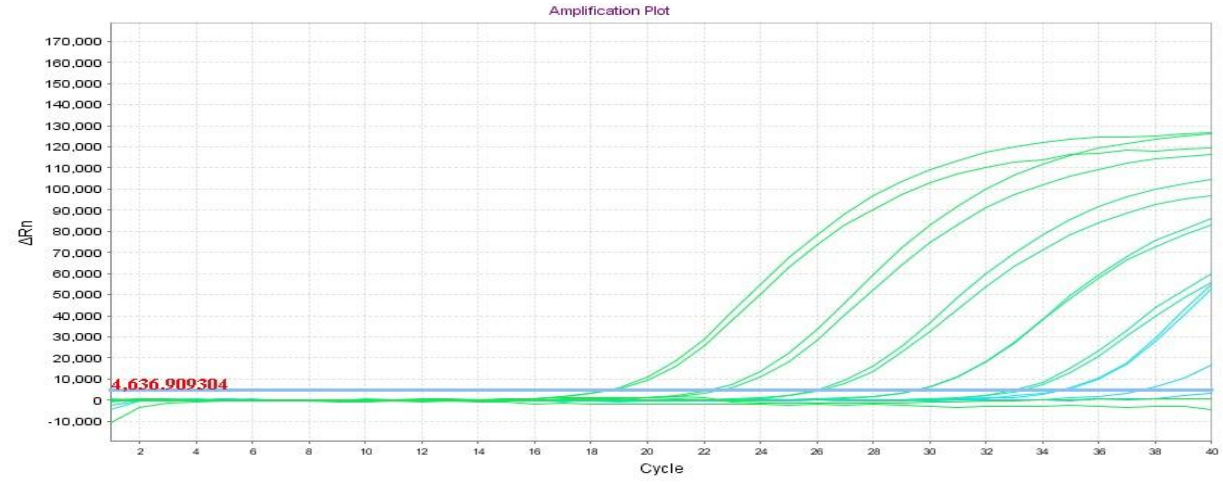
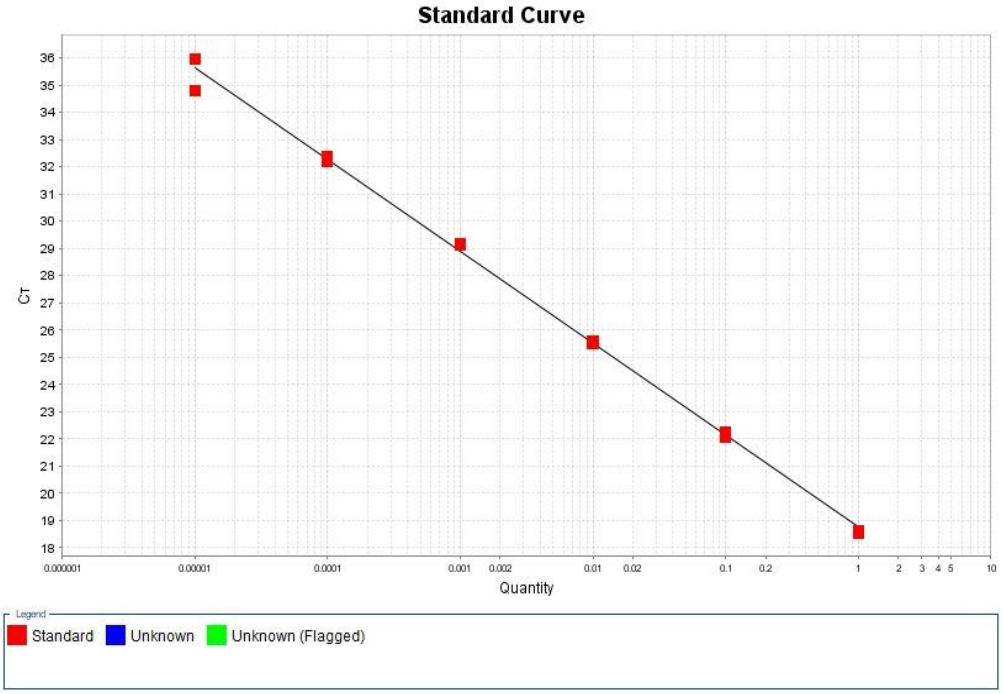


Figure S2_figure 1. The genus *Brucella* target standard curve. Efficiency (Eff%: 98.748, R^2: 0.956, y = -3.35x + 19.839

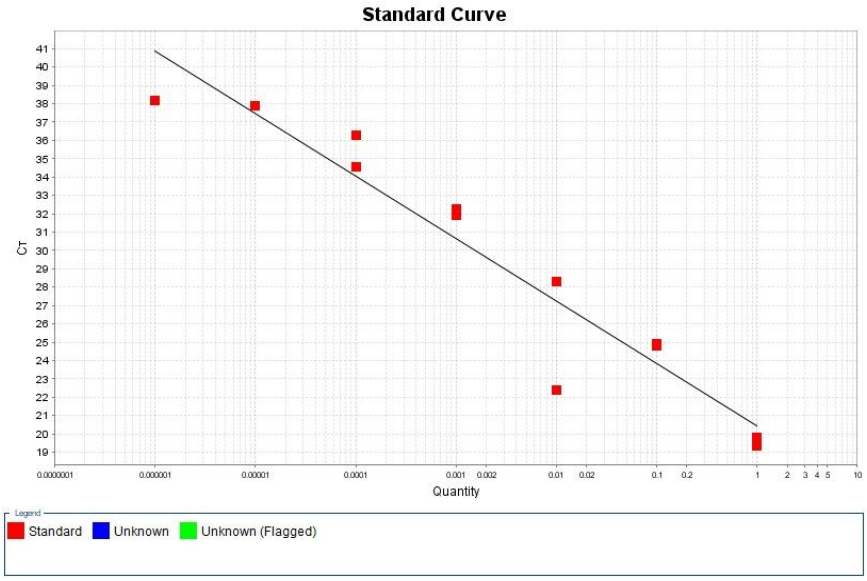
Standard curve for *B. abortus* controls





S2_figure 2: *B. abortus* amplification curve and standard curve. qPCR efficiency (Eff%: 98.004, R^2: 0.997, y=-3.371x +18.759)

Standard curve for *B. Melitensis*



S2_figure 3: B. melitensis standard curve. qPCR efficiency (Eff%: 96.448, R^2: 0.914, y=-3.41x +20.413)

3. Conclusion

The diagnostic assay for the detecting the genus *Brucella*, *B. abortus* and *B. melitensis* were optimal.

Appendix 3: Research Permit from NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/81438/29438** Date: **25th April 2019**

James Miser Akoko
International Livestock Research Institute
P.O. Box 30709 - 00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Host-pathogen association and transmission dynamics of Brucella in Kenya and Tanzania."*** I am pleased to inform you that you have been authorized to undertake research in **Isiolo, Marsabit and Narok Counties** for the period ending **25th April, 2020.**

You are advised to report to **the County Commissioners and the County Directors of Education, Isiolo, Marsabit and Narok Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Isiolo County

The County Director of Education
Isiolo County.

Appendix 4: Institutional Animal Care And Use Committee

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

CGIAR

3rd August 2018

Our Ref: ILRI-IACUC2018-16

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

Dear Roger Pelle & James Akoko,

REF: HOST PATHOGEN ASSOCIATION AND TRANSMISSION DYNAMICS OF BRUCELLA IN KENYA AND TANZANIA

I am pleased to inform you that International Livestock Research Institute Institutional Animal Care & Use (ILRI IACUC) has reviewed and approved your request to use animals in your research activity titled *'Host pathogen association and transmission dynamics of Brucella in Kenya and Tanzania'* as per the IACUC - ANIMAL USE FORM submitted on 12th July 2018 and subsequent amendments made on 20th July 2018 and 2nd August 2018.

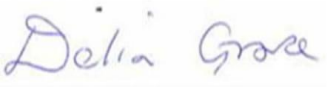
Please note that the approval is subject to compliance to the following:

- Compliance to applicable regulatory requirements and submission of documentary evidence;

- Minor changes required must be submitted to ILRI-IACUC for review and approval using the IACUC - ANIMAL USE MINOR AMENDMENT FORM before implementation.
- Only persons named on the approved IACUC - ANIMAL USE FROM (section 3 – 5) shall be allowed to handle and/or carry out sampling of the animals during this activity.
- Reporting of any adverse events to ILRI IACUC immediately; and
- Submission of completed PI Report Back Form upon completion of this activity to the ILRI IACUC.

Please call on ILRI IACUC on ILRIResearchcompliance@cgiar.org for any further clarification or information you may require.

Yours Sincerely,



Roger Pelle, PhD (pp. Delia Grace, PhD-IACUC)
Chair, ILRI Institutional Animal Care & Use Committee
Documents received & reviewed:

- IACUC - Animal Use Form
- Submitted amendments

Appendix 5: IREC Approval

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

CGIAR

9th August 2018

Our Ref: ILRI-IREC2018-14

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

Dear Roger Pelle & James Akoko,

REF: HOST PATHOGEN ASSOCIATION AND TRANSMISSION DYNAMICS FOR *BRUCELLA* IN KENYA AND TANZANIA

Thank you for submitting your request for ethical approval to the International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (IREC). ILRI IREC is registered and accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya, and approved by the Federalwide Assurance (FWA) for the Protection of Human Subjects in the United States of America.

I am pleased to inform you that ILRI IREC has reviewed and approved your study titled ***'Host pathogen association and transmission dynamics for Brucella in Kenya and Tanzania'***. The approval period is 9th August 2018 to 8th August 2019 and is subject to compliance to the following requirements:

- Only approved documents will be used;
- All changes must be submitted for review and approval before implementation;
- Adverse events must be reported to ILRI IREC immediately;
- Access and Benefits Sharing (ABS) requirements, where applicable;
- Submission of a request for renewal of approval at least 30 days prior to expiry of approval period; and
- Submission of an executive summary report within 90 days upon completion of the study.

Please call on ILRI IREC on ILRIResearchcompliance@cgiar.org for any further clarification or information you may require.

Yours Sincerely,

Silvia Alonso, PhD
Chair, ILRI Institutional Research Ethics Committee
Documents received & reviewed:

- Research Compliance Form & IREC Form
- Research Project Proposal
- Information Sheet and household Informed Consent Form
- Individual questionnaire for abattoir workers
- Individual human questionnaire

Appendix 6: Import permit

REPUBLIC OF KENYA
PHARMACY AND POISONS BOARD

Samples for Clinical Trials Import Permit

Document	321G - Samples for Clinical Trials Import Permit
Document Type	PER - Permits
Process	321G01 - Clinical trials permit process
Application Reference No : CD2018000PPB321G0001175505	Version No : 1
Master Approval No	Master Approval Version No
UCR Number	UCR201801311948

Application Status

Approval Status :AP - Approved	Used Status :	Application Date :20180911172534
Expiry Date :20190912	Amended Date :	Used Date :
Issuance Date :20180914		

Applicant Details

Name :INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

PIN :P051104412C	Application Code :ILR
Address :00	Country :KENYA
Contact Person :IVANMUHAVI	Email : I.Muhavi@cgiar.org

Consignee Details

Name :INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

PIN :P051104412C	OGA Ref No :
Physical Address :James Miser Akoko, International Livestock Research Institute ILRI Old Naivasha Road 00100 Nairobi, Kenya	Physical Country :KENYA
Postal Address :James Miser Akoko, International Livestock Research Institute ILRI Old Naivasha Road 00100 Nairobi, Kenya	Postal Country :KENYA
Telephone :4223000	Fax :4223001
Email :jamesakoko@yahoo.com	Sector of Activity :
Warehouse Code :	Warehouse Location :

Importer Details

Name :INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

PIN :P051104412C	OGA Ref No :
Physical Address :James Miser Akoko, International Livestock Research Institute ILRI Old Naivasha Road 00100 Nairobi, Kenya	Physical Country :KENYA
Postal Address :James Miser Akoko, International Livestock Research Institute ILRI Old Naivasha Road 00100 Nairobi, Kenya	Postal Country :KENYA
Telephone :4223000	Fax :4223001
Email :jamesakoko@yahoo.com	Sector of Activity :
Warehouse Code :	Warehouse Location :

Exporter Details

Name :Dr. Coletha Mathew

PIN :P000000000N	OGA Ref No :
Physical Address :Dr. Coletha Mathew Sokoine University P.O. Box 3000, Morogoro, Tanzania	Physical Country :TANZANIA
Postal Address :Dr. Coletha Mathew Sokoine University P.O. Box 3000, Morogoro, Tanzania	Postal Country :TANZANIA
Telephone :+ 255 23 2603511	Fax :+ 255 23 2640021
Email :sua@sua.ac.tz	Sector of Activity :Pharmaceuticals
Warehouse Code :DHL WORLDWIDE EXPRESS (K) LTD	Warehouse Location :JKA

Consignor Details

Name :Dr. Coletha Mathew

PIN :P000000000N	OGA Ref No :
Physical Address :Dr. Coletha Mathew Sokoine University P.O. Box 3000, Morogoro, Tanzania	Physical Country :TANZANIA
Postal Address :Dr. Coletha Mathew Sokoine University P.O. Box 3000, Morogoro, Tanzania	Postal Country :TANZANIA
Telephone :+ 255 23 2603511	Fax :+ 255 23 2640021
Email :sua@sua.ac.tz	Sector of Activity :Pharmaceuticals
Warehouse Code :DHL WORLDWIDE EXPRESS (K) LTD	Warehouse Location :JKA

Values - Header Level

Foreign Currency Code :USD	Forex Rate :100.82	FOB FCY :10.00
Freight FCY :0.00	Insurance FCY :0.00	Other Charges FCY :0.00
CIF FCY :10.00	FOB NCY :1,008.16	Freight NCY :0.00
Insurance NCY :0.00	Other Charges NCY :0.00	CIF NCY :1,008.16

Remarks

OGA Remarks :

1. ok
2. ok

Conditions Of Approval

1. ok

Purpose Of Import/Export

Research

Terms and Conditions

1.Ethical committee approval letter
2.Proforma Invoice/Invoice
3.PPB ECCTA

Item Details

Item No :1

Item Description :Serum Samples.
500 cattle samples in 2ml cryo tubes.

Item HS Code :3822001000	HS Description :TECHNICAL MATERIALS		
Quantity :500	Unit Of Quantity :tube	Supplementary - Quantity :0	Package Type :Tube
Package Quantity :500	Foreign Currency Code :USD	Unit Price FCY :0.01	Total Price FCY :5.00
Unit Price NCY :1.01	Total Price NCY :504.08	Country Of Origin :TANZANIA	Item Net Weight :500 tube
Item Gross Weight :500 tube	Applicant Remarks :For Research Purposes Only		

Item Details

Item No :2

Item Description :Milk Samples.
40 samples in 2ml cryo tubes.

Item HS Code :3822001000	HS Description :TECHNICAL MATERIALS		
Quantity :40	Unit Of Quantity :tube	Supplementary - Quantity :0	Package Type :Tube
Package Quantity :40	Foreign Currency Code :USD	Unit Price FCY :0.12	Total Price FCY :5.00
Unit Price NCY :12.60	Total Price NCY :504.08	Country Of Origin :TANZANIA	Item Net Weight :40 tube
Item Gross Weight :40 tube	Applicant Remarks :For Research Purposes Only		

Transport Details

Mode Of Transport :A	Mode Of Transport Desc :Air
Port Of Arrival :Jomo Kenyatta International Airport	Customs Office :JKA
Freight Station :Document Handling Limited	Cargo Type Indicator :Bulk

KEPHIS Specific Fields

Preferred KEPHIS Collection Office :Kephis Plant Quarantine Station-Muguga

Appendix 7: Ethical clearance from Tanzania

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: nimrethics@gmail.com

Ministry of Health, Community Development, Gender, Elderly & Children
University of Dodoma, College of Business Studies and Law
Building No. 11
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8a/Vol. IX/3102

23rd May, 2019

Coletha Mathew
Sokoine University of Agriculture
C/o Prof. Kazwala Rudovick
Sokoine University of Agriculture
P.O. Box 3021
Morogoro

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Approaches towards brucellosis control and prevention in Tanzania (Mathew C. et al), whose supervisor is Prof. Kazwala Rudovick of Sokoine University of Agriculture has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Morogoro, Mara, Arusha and Kilimanjaro regions.

Approval is valid for one year: 23rd May 2019 to 22nd May 2020.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

CC: Director, Health Services -TAMISEMI, Dodoma
RMO of Morogoro, Mara, Arusha and Kilimanajaro regions
DMO/DED of respective districts

Appendix 8: University approval letter

MASENO UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: PHD/PH/00102/016

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 18th July, 2018

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR JAMES AKOKO —PHD/PH/00102/016

The above named is registered in the Doctor of Philosophy in Biomedical Science Programme in the School of Public Health and Community Development, Maseno University. This is to confirm that his research proposal titled "Host-Pathogen Association and Transmission Dynamics of Brucella in Kenya and Tanzania" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

MASENO UNIVERSITY
18 JUL 2018
DEAN SCHOOL OF GRADUATE STUDIES

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES

Maseno University *ISO 9001:2008 Certified*

Appendix 9: Permission from the Counties

REPUBLIC OF KENYA

COUNTY GOVERNMENT OF MARSABIT

DEPARTMENT OF AGRICULTURE, LIVESTOCK AND FISHERIES DEVELOPMENT

DIRECTORATE OF VETERINARY SERVICES

Date 10.08.2018

RE: Consent for the study titled "Host Pathogen Association and Transmission dynamics of Brucella in Kenya and Northern Tanzania"

The above subject refers. Your study is quite relevant to the brucellosis disease situation in our County and it builds on to another study on Brucellosis that was done in the year 2013. I therefore wish to confirm to you that we are granting you the permission and consent to carry out your data collection in Marsabit for the purpose of the study.

Please note that while carrying out the study you are required to adhere to the ethical considerations and adequately liaise with my office and that of the Sub-county Veterinary Officer should you require any guidance.

We will offer you the necessary assistance that you require during the study.

Yours Faithfully

COUNTY DIRECTOR OF VETERINARY SERVICES
MARSABIT COUNTY
P.O.BOX 96-60500, MARSABIT

Dr. Boku Bodha

County Director Veterinary Services

Marsabit County

REPUBLIC OF KENYA

COUNTY GOVERNMENT OF MARSABIT

DEPARTMENT OF HEALTH

REPUBLICK OF KENYA

REF: CGM/HEALTH/GEN/Vol 1/117

17th, Dec , 2018

TO,

MR JAMES OKOKO

Email jamesakoko@gmail.com

Dear Mr. AKOKO

RE: RESEARCH "HOST PATHOGEN ASSOCIATION AND TRANSMISSION DYNAMICS FOR BRUCELLA iN KENYA AND TANZANIA "

I am in receipt of your email to undertake a PHD research in the above topic in Marsabit County that covers both Human and animal Health. I will like to highlight a fact that Marsabit County has the highest exposure to Brucellosis in both human and livestock population following reports of 2012 research by CDC and the Zoonotic Disease Unit of Ministry of Health, Kenya. Any further research in this field is highly welcome.

This office has no objection for you to undertake the research and gives you the go ahead with condition that all ethical considerations are upheld as stipulated in the attached ILRI – 1REC 2018 – 14 guidelines including Access and Benefit Sharing (ABS) requirements.

Dr Adano Diba Kochi

County Director

Preventive Promotive Health

Marsabit County

COUNTY DIRECTOR
PREVENTIVE / PROMOTIVE HEALTH
P. O. Box 5 - 60500, MARSABIT

NAROK COUNTY GOVERNMENT
DEPARTMENT OF HEALTH AND SANITATION

Telegrams: "HEALTH", Narok
Telephone: Narok 22300 and 22308
Fax: (050) 22394
Email: *countyhealthdirectornarok@gmail.com*

COUNTY DIRECTOR OF HEALTH
NAROK COUNTY
P.O. BOX 11- 20500
NAROK

When replying please quote our Ref and date

OUR REF: DIR/NRK CNTY/MOH/60/[154] **26th February, 2019**

All Medical Superintendents,
All Sub County Medical Officers of Health,
NAROK COUNTY

RE: RESEARCH AUTHORIZATION FOR JAMES MISER AKOKO ID/NO. 22366856

Reference is made to the ILRI letter Ref. No. ILRI-IREC2018-14 dated 9th August, 2018.

Authority is hereby granted to the above named to carry out research in Narok County for the period ending **8th August, 2019** on the topic ***'Host-pathogen association and transmission dynamics for Brucella in Kenya and Tanzania'.*** The research should be carried out in conformity with the study protocol and ethics.

Kindly accord the researcher the information and cooperation that he may require.

COUNTY DIRECTOR OF HEALTH
NAROK COUNTY GOVERNMENT
26 FEB 2019
P. O. Box 11-20500,
NAROK

Dr. Francis K. Kiio
County Director of Health
NAROK COUNTY

C.C. James Miser Akoko

NAROK COUNTY GOVERNMENT
DEPARTMENT OF HEALTH AND SANITATION

Telegrams: "**HEALTH**", Narok
Telephone: Narok 22300 and 22308
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Email: *countyhealthdirectornarok@gmail.com*

COUNTY DIRECTOR OF HEALTH
NAROK COUNTY
P.O. BOX 11- 20500
NAROK

When replying please quote our Ref and date

OUR REF: DIR/NRK CNTY/MOH/60/[154] **26th February, 2019**

All Medical Superintendents,
All Sub County Medical Officers of Health,
NAROK COUNTY

RE: RESEARCH AUTHORIZATION FOR JAMES MISER AKOKO ID/NO. 22366856

Reference is made to the ILRI letter Ref. No. ILRI-IREC2018-14 dated 9th August, 2018.

Authority is hereby granted to the above named to carry out research in Narok County for the period ending **8th August, 2019** on the topic ***'Host-pathogen association and transmission dynamics for Brucella in Kenya and Tanzania'.*** The research should be carried out in conformity with the study protocol and ethics.

Kindly accord the researcher the information and cooperation that he may require.

COUNTY DIRECTOR OF HEALTH
NAROK COUNTY GOVERNMENT
26 FEB 2019
P. O. Box 11-20500,
NAROK

Dr. Francis K. Kiio
County Director of Health
NAROK COUNTY

C.C. James Miser Akoko

Appendix 10: Consent forms

Host-pathogen association and transmission dynamics for *Brucella* in Kenya and Tanzania

Investigators:
Mr. James Miser Akoko, Maseno University, Afrique One-ASPIRE program and ILRI-BecA hub.
Prof. Collins Ouma- Maseno University.

Instructions

- **Enumerator to distribute read and explain to participant. Use English, Swahili, or local language, as appropriate.**
- **One signed copy for hardcopy file, one signed copy for participant.**

We are inviting you to participate in a research project, which is seeking to understand the transmission pathways for a bacterium called *Brucella*, that is known to cause disease in people and animals. People get infection when they consume raw contaminated livestock products, particularly milk or through contact with secretions from infected animals. The purpose of this study is to identify the species of *Brucella* responsible for causing infection in human and animals, and to give recommendations on the best strategies for controlling brucellosis in Kenya and Tanzania. To do this, we are requesting for your permission to allow us to administer a brief questionnaire and use your already collected blood samples for detection and identification of *Brucella* species. Findings from this investigation will help us know the most likely source of human brucellosis to advice policy makers on appropriate control strategies for this region. We will also communicate the results to you and advice on the control of brucellosis, should we detect *Brucella* from your blood sample. Information obtained during the interview will remain confidential to the research team and will only be used for the purposes of this project. All the data collected will be analysed by the main researchers and any confidential information will be kept private. The results of the study showing summaries only will be presented at national and international meetings and may be published for scientific purposes, without revealing your identity. You are free to choose not to be involved in the research and that even if you may have already consented to participate, you can refuse to answer any of the questions or stop participation at any time. In case you have any query, you can contact James Akoko on +254 722, 134 203 for further clarification.

Participant/authorized guardian statement

I confirm that I have understood the above description of the study and that I have had the opportunity to ask any questions about this study that I wish to ask. I confirm that I am happy to provide answers to the questions that will be asked of me and that I am happy to allow the project team to take the necessary samples for this project. I confirm that my samples may be stored and shipped as is necessary for the completion of this project and may be stored beyond the project for further medical research. I am aware that from the point of collection, I will not be personally identifiable; I understand that the project will not routinely report back the specific results of the tests to be carried out on my samples.

Participant name	Signature or thumb print	Date
Enumerator Name	Signature	Date

Appendix 11: Questionnaires

Individual questionnaire for hospital brucellosis sero-positive clients: *Brucella* host pathogen association and transmission dynamics project.

Note to the interviewer: Begin by introducing yourself (selves) and then the study background and objectives

This interview guide is designed to elicit information from you on the risk factors for transmission of brucellosis. Your participation in this study is highly valued and the responses you give will be used to inform interventions. All information you provide will be treated with utmost confidentiality.

Do you agree to be interviewed? YES ☐ NO ☐ *(if no, end interview)*

This questionnaire will be saved in a csv format and administered from a tablet

Questionnaire code:	**Start time (hour and minutes)**
Hospital identification code	Scan barcode
Individual identification code	Scan barcode

1. What is the name of the facility	
2. What is the gender of the study participant?	Male Female
3. How old are you?	Type in years
4. What is your location of residence	Type the name
5. We are also asking for your permission to allow us to retest your blood samples using PCR to identify the species of *Brucella*. This will give an idea of the possible source of your infection	Yes No
6. Blood sample available and permission granted? Yes/ No	Scan the barcode if yes.

Thank you for your participation

Finish time (hour and minutes)	

Gaafi nam sibitalat dukub anni iirat argani: brojecti ak wonni dukub annanitin dufatif namfud wolt hubanot barbath ta ammale kara iini thabrunile.

Qabsis nam wan kan gafatu: jalqab irrat nam uf barsis thuthubat wan baranot kanatif wan baranoti tau malef kara gababan

Karori qajeluma kunnin ta gaafi aka tin thaimtu kara dukubi anani naman senna tin himt barbath. Tanafu baranot kan kejirachu kanke gutho malt amale thebin atin nu thebift qarqars ath atha ka egeri kesat nu qarqar. Wonni atin nut himt chufti nukessa garu alatin himbat.

Ak sigafan nurra fudate? Eee ☐ Iyyo ☐ ☐ (yo iyyo jedan gaffi armumat oobat)

Gaafi tan kara mal ka csv jedamun midasani simle gugurthon gafatan

Lamba gaafin gargar basan:	**Saathi gaafin jalqabat (saatif thaqiqalle)**
Lamba sibital kana at kenit	Scan barcode
Lamba nam kana at kenit	Scan barcode

1. Maqan sibital kana man	
2. Nami gaafi tan gafachut jirt diir mo uww?	Diir ☐ Uww ☐
3. Ganni kanke hagam?	Ganna qor
4. Worri kanke garam	ollan qor
5. Ak dig kanke kan kara PCR jedamun lalan sikadan ak wan dukub kanan duf huban. Tunin kara dukubi kunin sitin duf nubesift	Eee ☐ Iyyo ☐
6. Diigilen yafudame amale ak lalan ya Eee jed? Eee/ Iyyo	Lamba arm kay ta qartasi gubba jir.

Galatom marro qumni tan nu wolin qothatef

Obati gaafi (saatif thaqiqale)	

Questionnaire for individual livestock: *Brucella* host pathogen association and transmission dynamics project.

Note to the interviewer: Begin by introducing yourself (selves) and then the study background and objectives

This interview guide is designed to elicit information from you on the risk factors for transmission of brucellosis. Your participation in this study is highly valued and the responses you give will be used to inform interventions. All information you provide will be treated with utmost confidentiality.

Do you agree to be interviewed? YES ☐ **NO** ☐ ***(if no, end interview)***

This questionnaire will be saved in a csv format and administered from a tablet

Questionnaire code:	**Start time (hour and minutes)**
Household identification code	Scan barcode
Animal identification code	Scan barcode

1. Which animal species do you have?	Cattle Sheep Goat Camel pigs
2. What is the species of this animal?	Cattle Sheep Goat camel
3. What is the sex of this animal?	Male Female
4. How old is this animal?	Type in years
5. Has this animal experienced any of the following syndromes?	Abortion Retained placenta Any other reproductive problem None of the above NA (if male)
6. Has this animal experienced any of the following syndromes?	Swollen testis Any reproductive problem None of the above NA (if female)
7. Blood sample available and permission granted? Yes/ No	Scan the barcode if yes.

Thank you for your participation

Finish time (hour and minutes)	

Gaafi hori matat gafatan: brojecti ak wonni dukub annanitin dufatif namfud wolt hubanot barbath ta ammale kara iini thabrunile.

Qabsis nam wan kan gafatu: jalqab irrat nam uf barsis thuthubat wan baranot kanatif wan baranoti tau malef kara gababan

Karori qajeluma kunnin ta gaafi aka tin thaimtu kara dukubi anani naman senna tin himt barbath. Tanafu baranot kan kejirachu kanke gutho malt amale thebin atin nu thebift qarqars ath atha ka egeri kesat nu qarqar. Wonni atin nut himt chufti nukessa garu alatin himbat.

Ak sigafan nurra fudate? Eee ☐ Iyyo ☐ ☐ (yo iyyo jedan gaffi armumat oobat)

Gaafi tan kara mal ka csv jedamun midasani simule gugurthon gafatan

Lamba gaafin gargar basan:	**Saathi gaafin jalqabat (saatif thaqiqalle)**
Lamba sibital kana at kenit	Scan barcode
Lamba nam kana at kenit	Scan barcode

1. Hori qencha kam fa qabth	Loon ☐ Olich ☐ Lales ☐ Gaal ☐ Booye ☐
2. Horin dig fudan kunin man?	loon ☐ olich ☐ lales ☐ gaal ☐
3. Hanchaf mo d'aala?	Hanchaf ☐ D'aala ☐
4. Gann hagam qab	Gannan qor
5. Wan akana kan irrat garte	salesa ☐ thillu itisa ☐ diib d'aal dow dibi ☐

	tokole ingar ☐ gaafin tun immalef ☐ (yo hanchaf tat)
6. Wan akana kan irrat garte	Kola ittaw fa ☐ Kukub quricho ☐ Ittes dibi ☐ gaafin tun immalef ☐ (yo d'ala tat)
7. Diigilen yafudame amale ak lalan ya Eee jed? Eee/ Iyyo	Lamba arm kay ta qartasi gubba jir.

Galatom marro qumni tan nu wolin qothatef

Obati gaafi (saatif thaqiqale)	