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Epidemiology of brucellosis in smallholder dairy cattle in Hai and Meru Districts, Northern Tanzania

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EPIDEMIOLOGY OF BRUCELLOSIS IN SMALLHOLDER DAIRY CATTLE IN HAI AND MERU DISTRICTS, NORTHERN TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Health and Biomedical Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Brucellosis is an endemic zoonotic disease in most developing countries caused by a facultative intracellular gram-negative bacterium of the genus Brucella. Brucellosis is one of the six priority zoonotic diseases in Tanzania with high social economic effects. A recently upsurge of brucellosis cases under the smallholder dairy cattle farming in many urban areas in Tanzania is a public health concern. A cross sectional study was conducted between January and June 2022 to establish the seroprevalence of brucellosis and possible risk factors in smallholder dairy cattle farming in the Hai and Meru Districts. To determine the seroprevalence, blood samples were analyzed for *Brucella* circulating antibodies using the Rose Bengal Plate Test and Competitive Enzyme Linked Immuno-Sorbent Assay. A structured questionnaire was presented to 200 smallholder dairy cattle farmers to explore the potential risk factors associated with brucellosis among dairy cattle in the study area. A total of 400 cattle were blood sampled from 10 villages in each district. The overall seroprevalence of bovine brucellosis was 0.50% and 0% for the Hai and Meru districts, respectively. Analysis of knowledge and management practices of brucellosis in the study areas showed that the majority of farmers (74.5%) knew the disease name; though, majority of them (87.9%) were not aware of the disease clinical signs. The indoor farming system mostly practiced in Hai and Meru District could have contributed to the observed low seroprevalence; thus, brucellosis free certification scheme can be implemented for continuous management of brucellosis in animals and humans as recommended by FAO.

DECLARATION

I, Peter Jiday Elisha do here declare to the senate of Nelson Mandela African Institution of Science and Technology that this is my original work and that it has neither been submitted nor being concurrently submitted for in any other higher learning institution.

Peter Jiday Elisha Date

The above declaration is confirmed by:

Prof. Gabriel M. Shirima Date

Dr. Esther G. Kimaro Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend the dissertation entitled "*Epidemiology of brucellosis among smallholder dairy cattle in Hai Meru Districts, Northern Tanzania*", in partial fulfilment of the requirements for the degree of Master of Science in Health and Biomedical Sciences of the Nelson Mandela African Institution of Science and Technology.

Prof. Gabriel M. Shirima Date

Dr. Esther G. Kimaro Date

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DEDICATION

To my lovely wife Ms. Anna Joshua Sumbe, my son Albrighton, my daughter Aubriella, my parents Mr. and Mrs. Elisha, my brothers Robert and Andrew, and the late grandfather Mr. Charles Subi Lupande Isomanga, who has made immeasurable contributions to my accomplishment in a variety of ways.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
c-ELISA	Competitive Enzyme Linked Immuno-Sorbent Assay
CFT	Complement Fixation Test
CI	Confidence Interval
CT	Coomb's Test
DED	District Executive Director
DVOs	District Veterinary Officers
FAO	Food and Agriculture Organisation
Fig	Figure
FPA	Fluorescence Polarisation Assay
HESLB	Higher Education Student's Loans Board.
IgG	Immunoglobulin G
IgM	Immunoglobulin M
KIDH-NM-AIST-CEDHA	Kibong'oto Infectious Disease Hospital-The Nelson Mandela African Institution of Science and Technology and the centre for Education Development in Health, Arusha
LFA	Lateral Flow Assay
LiSBE	Life Sciences and Bioengineering
mP	Milli-Polarization Units
MRT	Milk Ring Test
NM-AIST	Nelson Mandela African Institution of Science and Technology
OD	Optical Densities
ODK	Open Data Kit
OR	Odds Ratio
PCR	Polymerase Chain Reaction
QGIS	Quantum Geographic Inforamtion System
RBPT	Rose Bengal Plate Test
S19	Strain 19
SAT	Serum Agglutination Test
TVLA	Tanzania Veterinary Laboratory Agency
TZS	Tanzanian Shillings
URT	United Republic of Tanzania
USDA	United States Department of Agriculture

WOAH World Organisation of Animal Health

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

The smallholder dairy sector is among the growing livestock subsectors in Tanzania (Bingi & Tondel, 2015). This sector plays a great part in the national food security with its importance ranging from nutrition, health, employment opportunity; manure for farm fertilization, source of energy through biogas production; and generation of household income through selling of milk surplus and milk products (URT, 2015). Nevertheless, the dairy sector in Tanzania is constrained by a number of challenges, such as a seasonal change in pasture availability and quality, lack of broad-based dairy production technologies, low rate of milk processing, poor milk quality, poor milk handling facilities, long calving interval and diseases including zoonoses such as anthrax and brucellosis (Bingi & Tondel, 2015; Maleko *et al.,* 2018).

Brucellosis is among the endemic-zoonotic diseases of socio-economic importance in many regions and countries, such as Central Asia, South and Central America, Near East countries, India, Mexico, European Mediterranean countries and African countries, including Tanzania (Corbel, 2006). Globally, the frequence of occurance of brucellosis varies between 0% and 36% (Seleem *et al.,* 2010; Abu Sulayman *et al.,* 2020; Bodenham, 2020; Khurana *et al.,* 2021; Holt *et al.,* 2021; Ntivuguruzwa *et al.,* 2020; Wainaina *et al.,* 2020; Djangwani *et al.,* 2021; Mengele *et al.,* 2023). However, some European nations have eradicated the disease because of successful eradication and control programmes, including active surveillance coupled with test and slaughter policy and mass vaccination of domesticated animals (Seleem *et al.,* 2010). In Tanzania, the disease is among the six prioritized zoonotic diseases that require national attention. The endemicity of bovine brucellosis in Tanzanian dairy farming is attributed to a number of risk factors, such as inadequate surveillance of the disease, lack of vaccination of programmes, unrestricted animal replacement and movements, weak regulatory framework in culling of brucellosis-positive reactors with compensation funds from the Government, poor knowledge of the liverstock keepers on the disease, improper disposal of aborted fetus and retained placenta, animal interactions in grazing and watering points and breeding practices (Asakura *et al.,* 2019; Sagamiko, 2019; Bodenham *et al.,* 2020; Ntirandekura *et al.,* 2021; Katandukila *et al.,* 2021).

Brucellosis infection in smallholder dairy cattle causes not only negative socio-economic impacts but also public health consequences (Akakpo, 2009). Brucellosis control efforts in Tanzania in smallholder dairy farming system were effectively practiced in 1980-1990 and the prevalence was reduced to ≤ 2%. However, increase of the prevalence of bovine brucellosis among smallholder dairy cattle has increased from 0% - 22.1% has been reported in some areass of Tanzania after the collapse of the Animal Tuberculosis and Brucellosis control Program (Swai, & Kambarage, 2000; Karimuribo *et al.,* 2007; Mellau & Wambura, 2009; Shirima *et al.,* 2010; Swai & Schoonman, 2010; Shirima *et al.,* 2016; Bodenham, 2020; Mengele *et al.,* 2023). Although the smallholder dairy system in northern Tanzania is known to supply milk to major milk processing plants; little work has been done to ascertain the recent status of brucellosis in dairy cattle in these areas. Therefore, this investigated the disease status, spread, hot spots and possible disease determinants in smallholder dairy cattle in the Hai and Meru District Councils representing the Kilimanjaro and Arusha regions where smallholder dairy farming is highly prominent.

1.2 Statement of the problem

Although brucellosis is among the six prioritized zoonotic diseases in Tanzania with high socio-economic effects; its prevalence and associated risk factors in smallholder dairy cattle in Hai and Meru Districts is unknown. In 1928s the prevalence of brucellosis in smallholder dairy cattle farming in Tanzania was very high. However, effective implementation of the National tuberculosis and brucellosis control programme which was initiated between 1980-90 managed to reduce the prevalence of brucellosis to less than 2% (Shirima, 2005). Recently, there is an upsurge of brucellosis cases under the smallholder dairy cattle farming in Dar es salaam, Tanga, Morogoro, Arusha, Manyara and Njombe regions that creates public health concern (Swai & Schoonman, 2010; Shirima *et al.,* 2018; Mengele *et al.,* 2023). This has prompted further studies to clarify the magnitude of the disease spread and potential risk factors in areas prominent for smallholder dairy cattle farming such as northern Tanzania zone. The findings will enable appropriate interventions to be undertaken based on the current National Plan for Prevention and Control of Brucellosis in Humans and Animals (2018-2023).

1.3 Rationale of the study

Epidemiological data on bovine brucellosis provides scientific information for the timely intervention of the disease in the smallholder dairy farming subsector. This will help to encounter the socio-economic impacts of brucellosis in the value chain of dairy sector. The

results of this study will also provide an evidence-based analysis of the current status of brucellosis dissemination in the smallholder dairy cattle in northern Tanzania where dairy cattle farming is prominent. This study will also pave the way to understanding the spread, hotspots, and possible risk factors in northern Tanzania for sustainable control of the disease. Importantly, identifying hotspots and determination of the risk factors will help to understand options for prevention and control. Furthermore, the outcome of this study will pave the way for dairy farmers to consider brucellosis certification schemes as delineated in the Food and Agriculture Organisation (FAO) tactic for sustainable control of the disease in livestock and humans.

1.4 Research objectives

1.4.1 General objective

To establish the status of brucellosis prevalence and its associated risk factors among smallholder dairy cattle in Hai and Meru Districts.

1.4.2 Specific objectives

(i) To determine the seroprevalence of brucellosis among smallholder dairy cattle in Hai and Meru Districts.

(ii) To explore the risk factors for brucellosis in smallholder dairy cattle in Hai and Meru Districts.

1.5 Research questions

(i) What is the current seroprevalence of brucellosis among smallholder dairy cattle in Hai and Meru Districts?

(ii) What are the risk factors for brucellosis transmission among smallholder dairy cattle in Hai and Meru Districts?

1.6 Significance of the study

This research delivers evidence of the current seroprevalence of *Brucella* infection in smallholder dairy cattle in Hai and Meru Districts. This information will be useful for smallholder dairy cattle farmers to take the right actions such as the progressive slaughter of the positive reactors to cut off the transmission chain within and outside the herd. Also, the

determination of the possible risk factors for brucellosis transmission among smallholder dairy cattle will help to understand the appropriate interventions to impede the spread of the disease. This will prevent the impacts of brucellosis in animals such as abortion and still birth which reduces the stock size; cow sterility and chronic decrease in milk production. In addition to that, the smallholder dairy cattle farmers and animal product consumers will be safe from brucella infections thus the disease treatment costs in humans will be avoided.

1.7 Delineation of the study

The goal of this study was to establish the current status of brucellosis sero-prevalence and its determinants among the smallholder dairy cattle in Hai and Meru Districts for development of aproperiate control and prevention approaches. A cross sectional study involving blood collection from smallholder dairy cattle and questionnaire administration to dairy cattle farmers was conducted in Hai and Meru Districts, Northern Tanzania. The collected blood samples were processed to blood sera which were later on treated with RBPT and c-ELISA tests for detection of antibodies against *Brucella* infection. Also, this study has such limitation; the multi stage sampling design used in this study could have missed some infected farms, so some elements of purposive sampling should have been included.

CHAPTER TWO

LITERATURE REVIEW

2.1 Dairy cattle development

The dairy industry encompasses milk production and processing subsectors. Globally, there are over 270 million dairy cows according to 2020 USDA foreign agriculture service statistics. India has the largest number of dairy cows in the World (56 450 000) while Ethiopia is leading in Africa with more than 10 million dairy cows (Alemneh, 2019). Tanzania now has around 1 200 000 dairy cows as stated in the 2021 Ministry of Livestock and Fisheries' budget statement (URT, 2022). The Smallholder dairy cattle farms in Tanzania have a long history dating back to 1983, when the livestock policy was amended and the subsector was declared as one of the solutions to meet the country's milk demand (Kurwijila & Boki, 2003). Tanzanian small-scale dairy cattle farmers retain crosses of Tanzania Shorthorn Zebu with either Ayrshire, Jersey or Friesian due to environmental constraints (Swai & Karimuribo, 2011). The smallholder dairy subsector in Tanzania plays a great role in the national food economy with its importance ranging from nutrition, health, employment, manure, source of energy through biogas production and source of household income (URT, 2015). Despite of this huge potential, the dairy industry in Tanzania is limited by several factors including, seasonal changes in pasture availability and quality, inadequate broad-based dairy production technologies, poor milk values chain, reproductive wastage and diseases including brucellosis (Bingi & Tondel, 2015; Maleko *et al.*, 2018). Brucellosis is among the important zoonotic diseases in the country and prevalent in all livestock farming systems.

2.2 Brucellosis

2.2.1 Historical perspective of brucellosis

The chronicle of brucellosis traces back to 1887 when Micrococcus melitensis was first isolated in Malta by David Bruce from British soldiers' spleen samples whose death were suspect of undulating fever (Bruce, 1887 cited by Bodenham, 2020). A decade later, *Brucella abortus* was isolated from an aborted cow by a Danish veterinarian named Benhard Bang. In 1918, Alice Evans suggested the Brucella's name as a tribute to Sir David Bruce after finding the close relationship between Micrococcus meltensis and Abortus Bacillus of Bang (Seleem *et al.,* 2010). Several investigations have documented the occurrence of *Brucella* infection in both humans and animals worldwide. In Tanzania, the historical background of Brucellosis dated

back to early 1927 when the abortion outbreak in cattle was recorded in Arusha region (Kitali, 1984 cited by Shirima, 2005). From that period, the *Brucella* infection in animals has been established across the country albeit with variable magnitudes.

2.2.2 Aetiology of brucellosis

The causative agent of brucellosis is the gram-negative bacterium of the genus *Brucella* (Corbel, 2006). Although 12 species within the genus have been identified, the trio *Brucella* species namely *Brucella abortus, Brucella melitensis,* and *Brucella suis* are the common cause of brucellosis in both human beings and livestock (Dahouk *et al.,* 2017). It has been recognized that *B. melitensis* is the most potent brucellosis causative agent in human beings (Doganay & Aygen, 2003; Pappas *et al.,* 2008). However, the infected animal don't show clinical signs thus making the disease sometimes unnoticeable (Racloz *et al.,* 2013). The potential source of *Brucella* infections in human beings are domesticated animals (Corbel, 2006).

2.2.3 Distribution and prevalence of brucellosis in livestock in Tanzania

The investigations on brucellosis in animals were executed in several areas of Tanzania albeit with erratic prevalence in different livestock keeping systems. It has been reported that seroprevalence of brucellosis range from 0% to 22.1% in cattle, 5.1% to 11% in goats and 3.4% to 7.7 % in sheep (Karimuribo *et al.,* 2007; Mellau & Wambura, 2009; Shirima *et al.,* 2010; Swai & Schoonman, 2010; Shirima *et al.,* 2016; Bodenham, 2020; Mengele *et al.,* 2023) (Table1).

Table 1: Seroprevalence of brucellosis in various livestock system in Tanzania

Location	Farming systems	Study design	Species	Sample size	Seroprevalence	Diagnostic test	References
			Small ruminant	125	0		
Sumbawanga	Pastoral and small holder	Cross sectional	Cattle	354	0.8	RBT and cELISA	Maengo (2017)
Bukombe	pastoral, Agro pastoral and smallholder	Cross sectional	Cattle	221	1.4	RBT and cELISA	Makoye (2017)
			Goats	1892	5.1		
			sheep	1739	3.4		
Arusha	smallholder	Cross sectional	cattle	318	0.3	c-ELISA	Mengele *et al.* (2023)
Kilimanjaro	smallholder	Cross sectional	cattle	521	2.5	c-ELISA	Mengele *et al.* (2023)
Iringa	smallholder	Cross sectional	cattle	281	0.4	c-ELISA	Mengele *et al.* (2023)
Njombe	smallholder	Cross sectional	cattle	187	15.5	c-ELISA	Mengele *et al.* (2023)
Tanga	smallholder	Cross sectional	cattle	523	1	c-ELISA	Mengele *et al.* (2023)
Mbeya	smallholder	Cross sectional	cattle	217	0	c-ELISA	Mengele *et al.* (2023)

2.2.4 Transmission of brucellosis between animals and associated risk factors

Animal-to-animal spread of brucellosis can happen once animals get in touch with either infected aborting cows, aborted fetus, secretions from the infected animals, contaminated grazing areass, or contaminated animal pens after parturition. Moreover, artificial insemination using semen from infected bulls has been reported to transmit brucellosis (Osoro *et al.,* 2015; Bodenham, 2020). Furthermore, transmission can happen through consuming contaminated feeds, pasturelands, water or drawing the tongue over infected foeti, placenta, or reproductive organs of infected cow shortly after miscarriage or regular parturition (Corbel, 2006). Furthermore, transmission may occur rarely through inhalation or via conjunctiva (WHO, 2006). Some husbandry practices such as communal grazing, animal replacement, herd size, vaccination levels and farming systems have been reported to influence the risk of transmission of the disease agent (Corbel, 2006).

Brucellosis in smallholder dairy cattle can be attributed by numerous factors such as a lack of knowledge on brucellosis and improper livestock management and practices. It has been reported that some dairy farmers do not understand brucellosis transmission dynamics-and how the disease can be prevented or controlled. Many researchers have pointed out that illiterate among farmers on brucellosis is associated with seropositivity of their livestock (Sijapenda *et al.,* 2017; Asakura *et al.,* 2019; Shirima *et al.,* 2018; Ismail *et al.,* 2019; Ntivuguruzwa *et al.,* 2020; Ntirandekura *et al.,* 2021; Mengele *et al.,* 2023). Animals that grazed on free range system have been reported to have higher seropositivity compared to that of zero grazing system. Animal interaction at either grazing or water points is more pronounced in free range systems compared to zero grazing system (Ntivuguruzwa *et al.,* 2020). Unreliable animal source jeopardizes the health of dairy cattle due to higher possibility of introducing infected animals in the farm. The downfall of the Tuberculosis and Brucellosis Control Programme as well as the fade of parastatal farms that supply heifers to farmers has reported as one the reasons for spread of brucellosis among dairy farmers in Tanzania (Shirima *et al.,* 2018). Another determinant of brucellosis in small-scale dairy farms that has been noted by numerous researchers is a reproductive technique. Breeding dairy cattle by using a bull is associated with seropositivity in dairy cattle grazing systems when compared with artificial insemination (Sijapenda *et al.,* 2017). Improper disposal method of an aborted foetus and retained placenta fuel transmission of brucellosis pathogens in dairy cattle (Asakura *et al.,* 2019; Ntivuguruzwa *et al.,* 2020).

2.2.5 Clinical manifestation of brucellosis in livestock

In livestock, the incubation period of brucellosis shows a great variation from one animal species to another but even within species. The variability in clinical signs is governed by a many factors such as the immunity of the animal, the magnitude of infective dose, age and sex of the animal as well as the gestation period (Corbel, 2006). Clinical findings in livestock include abortion especially during the last trimester, reduced milk production, metritis, long calving interval, retained placenta, infertility that can occur in both bovine, caprine, ovine, swine and canine while inflammation of the seminal vesicles, hygromas and orchitis are very common in male animals (Corbel, 2006).

2.3 Diagnosis of brucellosis

At present, the recognition of brucellosis is achieved through clinical examination, which is supplemented by either serological testing, molecular methods, or microbiological culture (Corbel, 2006). Some techniques are designed basically for either detection of Brucella presence within the sample or the detection of specific antibodies (Ducrotoy *et al.,* 2017). There is a broad variety of serological tests created for the identification of antibodies as the host's immune response against Brucella infections. These indirect tests are: Fluorescence Polarisation Assay (FPA), Enzyme Linked-Immunosorbent Assay (ELISA), Milk Ring Test (MRT), Serum Agglutination Test (SAT), Complement Fixation Test (CFT), Coomb's test (CT), Rivanol Precipitation Test (RvPT) and Rose Bengal Plate Test (RBPT) (Corbel, 2006). The creation of a Competitive ELISA (c-ELISA) with very high sensitivity and specificity has been a very useful tool to distinguish naturally infected animals and vaccinated ones (Bodenham *et al.,* 2021). Microbiological culture, Microscopic examination and molecular techniques have been used as direct testes for detecting Brucella organism in the host's sample (Corbel, 2006).

2.3.1 Diagnostic tests to demonstrate the presence of Brucella pathogens

(i) Culture

Isolation and culturing of the microbe depend on the ideal selection of the sample from the animal. In clinical cases the samples can be taken from the following: Vagina discharge, aborted foetus (spleen, lungs and stomach), semen, fluid from hygroma, milk and colostrum. In acute/chronic conditions the samples can be taken from the genital and oropharyngeal lymph nodes, mammary glands as well as from the spleen (Corbel, 2006; Alton, 2019). Farrell

medium is the mostly used medium because it has antibiotics that limit the growth of other bacteria. The cultured bacteria are expected to grow after 2-3 days while the negative results can be recorded after 2-3 weeks of incubation (Corbel, 2006). Culturing method has many limitations including the following: infection risk to the laboratory workers and slow growth rate thus time-consuming (Corbel, 2006; Smirnova *et al.,* 2013).

(ii) Microscopic examination

After culturing, *Brucella* organism can be identified through Stamp's modified Ziehl Nelsen staining. Through this staining, they appear red colour small, singly or paired coccobacilli. However, the same colour can be expressed by *Coxiella*, *Chlamydia* and *Norcardia specie*s (Corbel, 2006; Smirnova *et al.,* 2013; Alton, 2019) and thus, complicates microscopy examination in areass where these pathogens are prevalent.

(iii) Molecular technique

The use of Polymerase Chain Reaction has been documented as one of the very promising molecular techniques that have high sensitivity and specificity with the ability to detect the least infectious dose of less than 5 Brucella in the sample. Moreover, PCR technique is neither affected by cross-reaction nor disease stage. However, apart from the need of skilled personnel to run the PCR machines, the high costs of primers specific for each Brucella species are the limitations of the use of the technique (Corbel, 2006; Khan & Zahoor, 2018; Alton, 2019) in developing countries.

2.3.2 Serodiagnosis tests for brucellosis

There are a number of recognised standard serological assays for antibodies detection in animals. These are: Serum Agglutination Test (SAT), Rose Bengal plate test (RBPT), Complement Fixation Test(CFT), Enzyme Linked -Immunosorbent Assay (ELISA), Lateral Flow Assay (LFA) and Fluorescence Polarization Assay (FPA) (Corbel, 2006; Nielsen, 2010).

(i) Serum Agglutination Test (SAT)

This is among the widely used standard method of antibodies detection in animals. Nevertheless, the test has some limitations such inability to identify antibodies after miscarriage or during the initial period of disease development and inability to distinguish natural infection from vaccination with S19 (Poester *et al.,* 2010). Apart from that, SAT has

a poor specificity because of IgM interactions with other bacterial species (Corbel, 2006; Alton, 2019).

(ii) Complement Fixation Test (CFT)

The CFT is regarded as a definitive serodiagnosis method since possesses good specificity and sensitivity. The test is also used as a confirmatory test though has been replaced by FPA and cELISA (Alton, 2019). The test is not cross reacted by antibodies produced following S19 vaccination in animals. However, the test is costly, time-consuming, complex, and require skilled personnel (Corbel, 2006).

(iii) Rose Bengal Plate Test (RBPT)

The RBPT is fast, simplest and mostly used serodiagnosis test for screening brucellosis in animals. The RBPT is the spot agglutination test which use PH 3.65 buffered antigens. Buffering reduces cross-reactions by enhancing IgG agglutination and prevention of IgM agglutination (Corbel, 2006; Alton, 2019). Through this test, the serum is blended with the dyed antigen droplets on a visible plate and the positive reaction is signified by any agglutination results. Despite of cheapness and simplicity of the test; it can generate false positive results in vaccinated animals because of the low specificity and high sensitivity hence needs to be confirmed by other robust tests. In addition to that the test can be interfered by cross reactions from other bacteria such as *Vibrio cholerae, Escherichia coli, Yersinia enterocolitica,* and *Salmonella landau* (Corbel, 2006; Alton, 2019). Another limitation of using RBPT is it's poor performance on chronic brucellosis-infected animals as it mainly detects IgM, yet the amount of IgM in chronic infected animals diminishes with time (Nielsen, 2010; Teng *et al.*, 2017; Alton, 2019).

(iv) Enzyme Linked-Immunosorbent Assay (ELISA)

The ELISA is a serodiagnostic test which offers excellent specificity and sensitivity compared to other serological tests. There are two categories of ELISA namely competitive (c-ELISA) indirect (i-ELISA) (Nielsen, 2010). The i-ELISA works on the principle of binding the antibody present in the serum with its antigen leading to the formation of the immune complex which is then detected by using a marker molecule (Nielsen, 2010; Alton, 2019). The test mostly uses species-specific enzyme-labelled conjugates for each species under test. Despite of having high sensitivity; i-ELISA has low specificity since can't differentiate naturally infected animals from S19 vaccinated thus regarded as a screening test. Furthermore, the test

is affected by cross reaction from other bacteria thus false positive results were reported (Nielsen 2010). Following the weakness of i-ELISA; the superb c-ELISA test with high sensitivity and specificity of 95.2% and 99.7% respectively was developed (Godfroid *et al.*, 2010; Etman *et al.*, 2014). Compared to i-ELISA; c-ELISA is simple to execute, robust, encounter the limitation of low specificity, can distinguish the naturally infected animals from vaccinated ones, is not affected by cross-reacting bacteria and can be applied in areas with low seroprevalence of brucellosis; for these case, the test is used as a confirmatory test (Corbel, 2006; Nielsen, 2010; Alton, 2019). Also, the test can detect all antibody isotypes (IgM, IgG1, IgG2 and IgA) thus suitable for brucellosis diagnosis in either acute or chronic infected animals. However, the test is a bit complicated and more expensive compared to other serological tests (Nielsen, 2010).

(v) Lateral flow assay (LFA)

This is the immunochromatographic assay designed for detection of antigen-specific in the animal serum sample. The test is regarded as a simplified ELISA due to its ability to bind to specific antibodies present in the serum sample to an antigen fixed on the test strip employing a secondary conjugated antibody to visualize. It is one of the simple and rapid brucella screening methods that does not require experienced experts, electricity and advanced equipment. It has 90% and 100% sensitivity and specificity in animals (Alton, 2019).

(vi) Fluorescence Polarisation Assay (FPA)

This is the rapid and simple homogeneous assay technique for the detection of antibody/ antigen interaction and can be performed either in the field or laboratory. The technique requires special reagents and reading tools (Corbel, 2006). The principle of this technique is the correlation between the rotational speed of a molecule in the liquid and its mass. The rotational speed of molecules is inversely related to their masses. Therefore, smaller molecules are faster and depolarize beams of more polarized light than larger molecules. In the test, the magnitude of depolarization is measured in units of millipolarization (mP). Serum samples were incubated with fluorescein isothiocyanate labeled with the specific antigen Brucella spp (Alton, 2019). In the positive serum sample, there is a formation of large fluorescent complexes while the antigen remains uncomplexed in the negative sample. The sensitivity and specificity of FPA are more or less the same as that of cELISA. The test can eliminate the effect of cross reactions and is capable of differentiating S19 vaccination from natural infections (Nielsen, 2010; Alton, 2019).

2.3.3 Management of brucellosis in livestock

Control and eradication measures of Brucellosis in animals are highly dependent on numerous factors such as national strategies, policies and priorities (Corbel, 2006). However, WOAH provides the guidelines and strategies for control and eradication which have been useful in many countries (Corbel, 2006). Mass immunization (vaccination), test and slaughter of infected animals, restriction of movements of suspected and infected animals to prevent the disease spread between animals and training programmes are some of the common strategies deployed in most countries (Corbel, 2006) to prevent the endemicity of brucellosis. Furthermore, in the year 2018. The Tanzanian Ministry of Livestock and Fisheries Development created a National Plan for the management of Brucellosis in Humans and Cattle. The strategies outlined in the National Brucellosis control plan are vaccination, one health approach surveillance on brucellosis, sensitization to livestock keepers on proper disposal method of an aborted foetus, placenta and its fluid and observing good animal husbandry practices were highlighted and emphasized. Some vaccines used for immunization against brucellosis include RB 51 and S19 (Corbel, 2006). In Tanzania, the use of S19 vaccine was adopted and confined to the Government and parastatal dairy Cattle in the early 1980s (Kambarage personal communication, 2003 cited by Shirima, 2005). The S19 is the widely used vaccine in cattle without conferring immunity across other species. Furthermore, there is a lack of vaccination campaigns against brucellosis in Tanzania (URT, 2018). At present, there is no operative medication of brucellosis in animals as some problems such as creation of carrier animals which affects future serological detection have been reported due to the use of antibiotics like penicillin and oxytetracycline (Khan & Zahoor, 2018). Also, the cost of treatment does not favour the treatment option in cattle.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Study areas

This research was conducted in Hai and Meru Districts in northern Tanzania (Fig. 1). The two districts were sampled according to large population of smallholder dairy cattle farms. The Hai District is located between the latitudes of 20 50' and 30 29'S south of the Equator, and the longitudes of 300 30' and 370 10' to 380E, with typical temperatures ranging from 25°C to 32°C during the dry season. To the north, the Rombo District and the Mt. Kilimanjaro National Park, to the west side Siha and Arumeru Districts, to the south, Simanjiro District, and to the east, Moshi District. Both short and long rain seasons occur in the District. The Hai District is subdivided into three divisions, 17 Wards and 62 Villages. It has a total of 49 316 dairy cattle and occupies an area of 1011 square kilometres (101 100 ha). Meru District Council on the other hand, is located in the Eastern South of the Equator, between Latitude 3'000 – 3'400S and Longitude 360 – 5500E; annually, the rainfall and temperatures range from 500 mm to 1200 mm and 17°C to 32°C, respectively. The size of the District is 1268.2 km^2 and is administratively divided into 3 divisions, 26 wards, and 90 villages. The district has a total of 98 001 dairy cattle (Fig. 1).

3.2 Study population

In this study, dairy cattle of at least one year old under the smallholder farming system were recruited. Also, household heads or someone knowledgeable with the herd and aged above 18 years old were interviewed on knowledge and management practices allied with brucellosis control.

3.3 Study design

A cross sectional study design was applied.

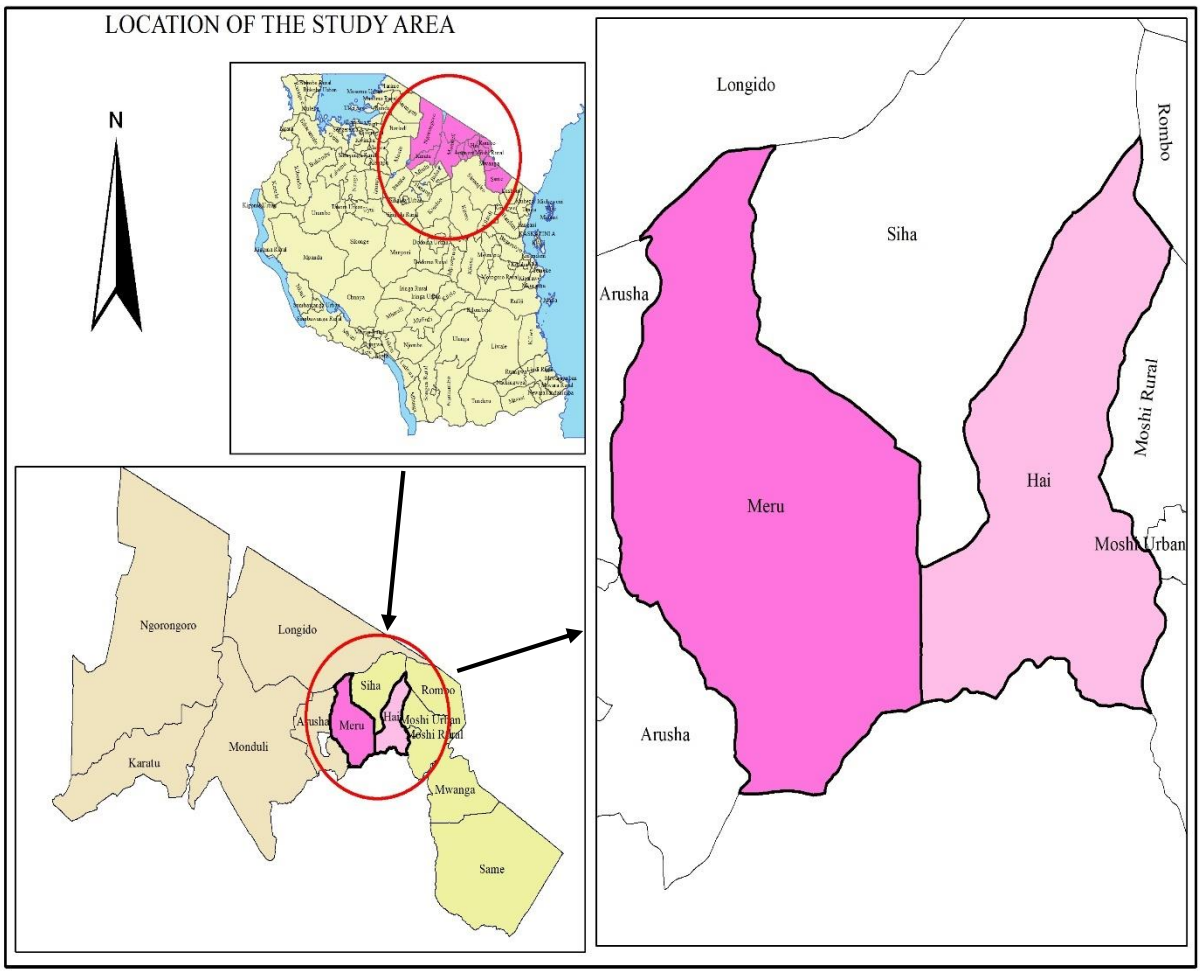

Figure 1: The map of the study areas showing the boundaries of Meru and Hai Districts (Created by using QGIS)

3.4 Sample size estimation and sampling procedure

The sample size was obtained using Fisher's (1998) statistical procedure, with a 95% confidence interval, 5% margin error and an estimated prevalence of 50% in smallholder dairy cattle. The estimated prevalence of 50% was used in this study as there was no previous study conducted in the study areas with its focus on smallholder dairy cattle. The following formula was used for the sample size calculation:

$$N = \frac{Z^2 * p(1-p)}{c^2}$$

Where:

N = Sample size,

P = Estimated prevalence = 0.5,

Z = Level of confidence as 1.96 and

c = Desired precision level = 0.05.

The calculated minimum sample size was 384, even so, the sample size "N" used in this research was 400 smallholder dairy cattle for homogenity of sampling in each herd. A multistage sampling approach comprised of 62 Villages and 17 Wards in Hai District while that of Meru District comprised of 90 Villages and 26 Wards. Firstly, there was a purposive selection of 5 wards with a large number of dairy cattle from each District according to information provided by the District Veterinary officers (DVO); proceeded by a random sampling of two villages representing each ward in which 20 dairy cattle from 10 dairy cattle-keeping households (2 dairy cattle per household) were selected randomly for blood sampling. In this study, the selected households were regarded as primary sampling units. The 200 household heads were scrutinized for determination of the potential risk factors for brucellosis spread among smallholder dairy cattle. At every stage of random sampling, run if () function in statistical software R was deployed.

3.5 Field data collection

3.5.1 Research clearance and ethical consideration

The research project was approved by Kibong'oto Infectious Disease Hospital-The Nelson Mandela African Institution of Science and Technology and the centre for Education Development in Health, Arusha (KIDH-NM-AIST-CEDHA)-KNCHREC with certificate number KNCHREC0067/04/2022 issued on 27th June 2022 (Appendix 3). Also, research permission was provided by the respective District Executive Directors (DEDs) in response to the introduction letters from NM-AIST (Appendix 4 and 5). The eagerness of the household heads to be involved in this study was sought through written consent before execution of the study.

3.5.2 Blood collection and processing

Cattle were restrained manually to avoid harm or any causes of animal discomfort during sample collection. The exercise was done in compliance with the Tanzania Animal Welfare Act, part V (Animal Welfare Act 2008). Using a halter, the animal's head was fastened to an elevated position to allow the jugular vein easily tracked and pinched. Then thumb finger was pressed at the base of the jugular groove to raise blood pressure and visualize the vein by blocking the vein. With plain vacutainer and needle; 10 ml blood was drawn from the vena jugularis of each restrained animal. Each animal was identified according to the identity type provided by the owner for subsequent identification. This enables vacutainer tubes containing

blood samples being labelled accordingly. To prevent coagulation of the albumin, the collected blood samples were kept at 25°C for about 30 minutes before centrifugation. Centrifugation was done at Tanzania Veterinary Laboratory Agency's (TVLA) Laboratory-northern zone office, Arusha where the vacutainer tubes were spun at 3000 rpm for 10 minutes (BHG S Segurita-Germany). After centrifugation, the tubes were opened and the formed sera were emptied into 2.0 ml cryogenic vials. The sera were stored temporarily at TVLA laboratory at approximately -20°C soon after separation before transfer to the Nelson Mandela African Institution of Science and Technology's (NM-AIST) laboratory for analyses. Likewise, at NM-AST laboratory the sera were stored at -20°C.

3.5.3 Assessment of risk factors for brucellosis transmission

The structured survey comprised both open and closed ended questions which was coded by using an Open Data Kit (*https://opendatakit.org*) mobile application. The questionnaire covered a wide range of information on brucellosis knowledge and management practices of brucellosis this include questions on general understanding about bovine brucellosis and clinical signs, the use of vaccines, the use of veterinary services, herd management practices, herd size, abortion cases, recent reproductive status, retained placenta, handling of aborting cows and aborted foetus, heifer sources, breeding methods, grazing system, milk production trends, past two calving dates, livestock movement and interaction with neighbouring cattle herds as well as milk distribution channels, price and value chain (Appendix 1). Pre-testing of the questionnaire was done to smallholder dairy cattle farmers in Monduli District Council before development of the final version. During field visit the questionnaire was administered to the respondents (head of household or someone knowledgeable with the herd and above 18 years old). It took about 25-30 minutes to complete the questionnaire successfully.

3.5.4 Laboratory analysis

Laboratory analysis was carried out at the NM-AIST laboratory where all 400 serum samples were screened using the RBPT while the positive reactors were confirmed by using cELISA. The detailed procedures for the performed laboratory tests are as described below:

(i) The Rose Bengal plate test (RBPT)

The 400 sera were tested for Brucella antibodies using the Zoetis™ Brucella Rose Bengal test kit from Delpharm Biotech (Lyon Cedex 07-France). Using an applicator stick, equal proportions (30 µL) of the investigated serum and antigen were completely mixed on the glass

plate in accordance with the producer's instructions. The plate was then gently rocked to ensure thorough mixing. The plates were visually examined for agglutination after four minutes in comparison to a positive control (Fig. 2). Any level of agglutination was noted as positive result, however the absence of agglutinations was taken as negative result. In an Excel spreadsheet, the results were noted and stored. The white glass plate was cleaned with methylated alcohol and clean water, then allowed to dry for around 5 to 10 minutes before being used again.

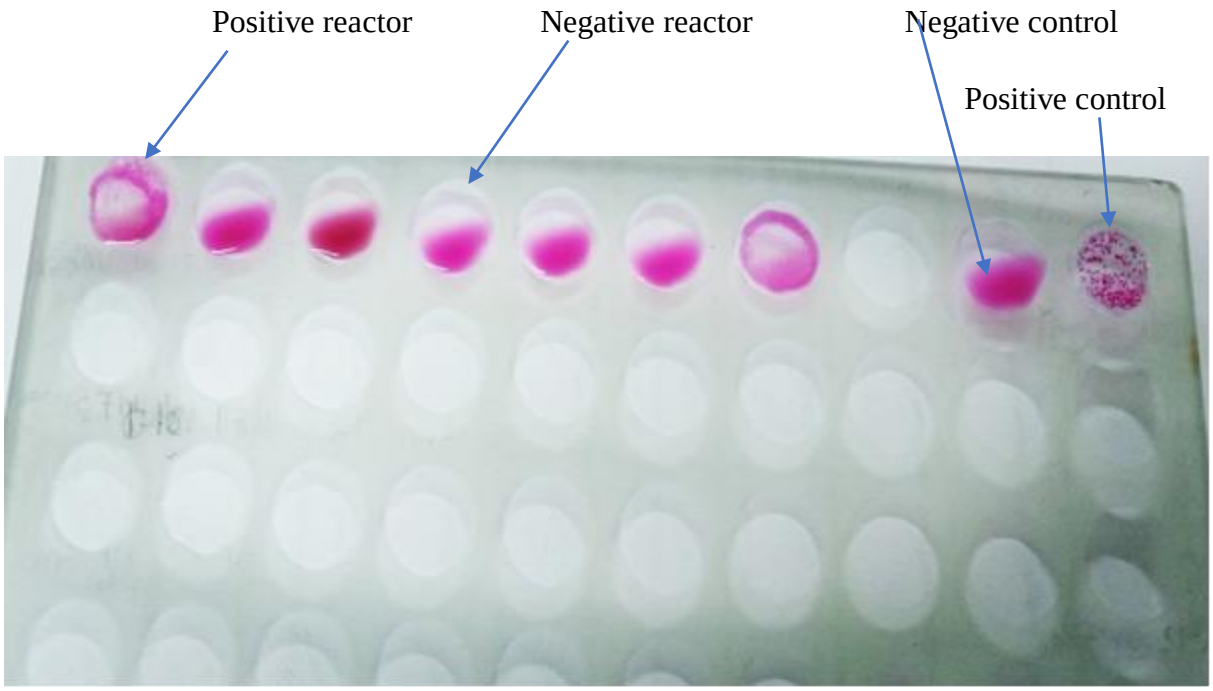

Figure 2: The Rose Bengal Plate view

(ii) competitive Enzyme-Linked Immunosorbent Assay (cELISA)

The serum sample that reacted positive to RBPT was tested by cELISA according to (COMPELISA) APHA SCIENTIFIC of the United Kingdom. The positive reactor sample was combined with 39 randomly selected serum samples that reacted negatively on RBPT to make a single full microtitre plate during cELISA test. Briefly, a 25^{o}C water bath was used for warming the diluting buffer solution before its application. The conjugate mixture was then prepared and diluted to working concentration. Columns 11 and 12 served as controls before 20 µL of each test serum were added in triplicate to each well. Wells F11, F12, G11, G12, H11, and H12 each received 20 µL of a positive control. Wells A11, A12, B11, B12, C11, and C12 each received 20 µL of a negative control. There was nothing added in columns 11 and 12 thus acted as conjugate control (D11, D12, E11 and E12) (Fig. 3). We immediately dispensed 100 µL of the produced conjugate mixture into wells to achieve a final serum dilution of 1/6. The plate was vibrated slowly for 30 seconds with the lid on, followed by 10 seconds of careful

hand shaking every 10 minutes for an hour. Before washing the plate five times with clean water under low pressure from a faucet, the plate was shaken to remove its constituents. When no more liquid was visible, the plate was thoroughly dried by firmly tapping it on several layers of absorbent paper towels. One urea H_2O_2 tablet was liquified in 12 ml of distilled water to create the OPD solution. The OPD pill was added and properly blended once it had dissolved. The prepared OPD solution was then kept in an opaque container to avoid exposure to light. All wells received 100 µL of OPD solution, and the plate underwent a 20-minute incubation period at room temperature. At intervals of five minutes, the plate was very carefully and lightly tapped. After turning on the microplate reader, the apparatus was given 10 minutes to stabilize. By adding 100 µL of stopping mixture to each well, the reaction was delayed. A paper towel that was absorbent was used to wipe away the moisture from the plate's bottom. The optical densities (OD) of the samples and controls were measured at 450 nm in less than 10 minutes. 60% of the mean of the OD of the four conjugate control wells was used to determine if a value was positive or negative. Any test sample with an OD equal to or lower than this value was considered positive. The following were the acceptance criteria of the plate as recommended by the manufacturer:

(i) The six negative control wells' average OD should be higher than 0.700 (the ideal mean negative OD is 1.000).

(ii) The six positive control wells' mean OD should be under 0.1.

(iii) The ideal mean conjugate control value is 1, and the conjugate control wells' mean OD should be higher than 0.700.

(iv) The binding ratio should be greater than 10.

The binding ratio was calculated as:

$$\boldsymbol{Binding\ ratio = \frac{Mean\ of\ 6\ negative\ control\ well}{Mean\ of\ 6\ postive\ \ control\ wells}}$$

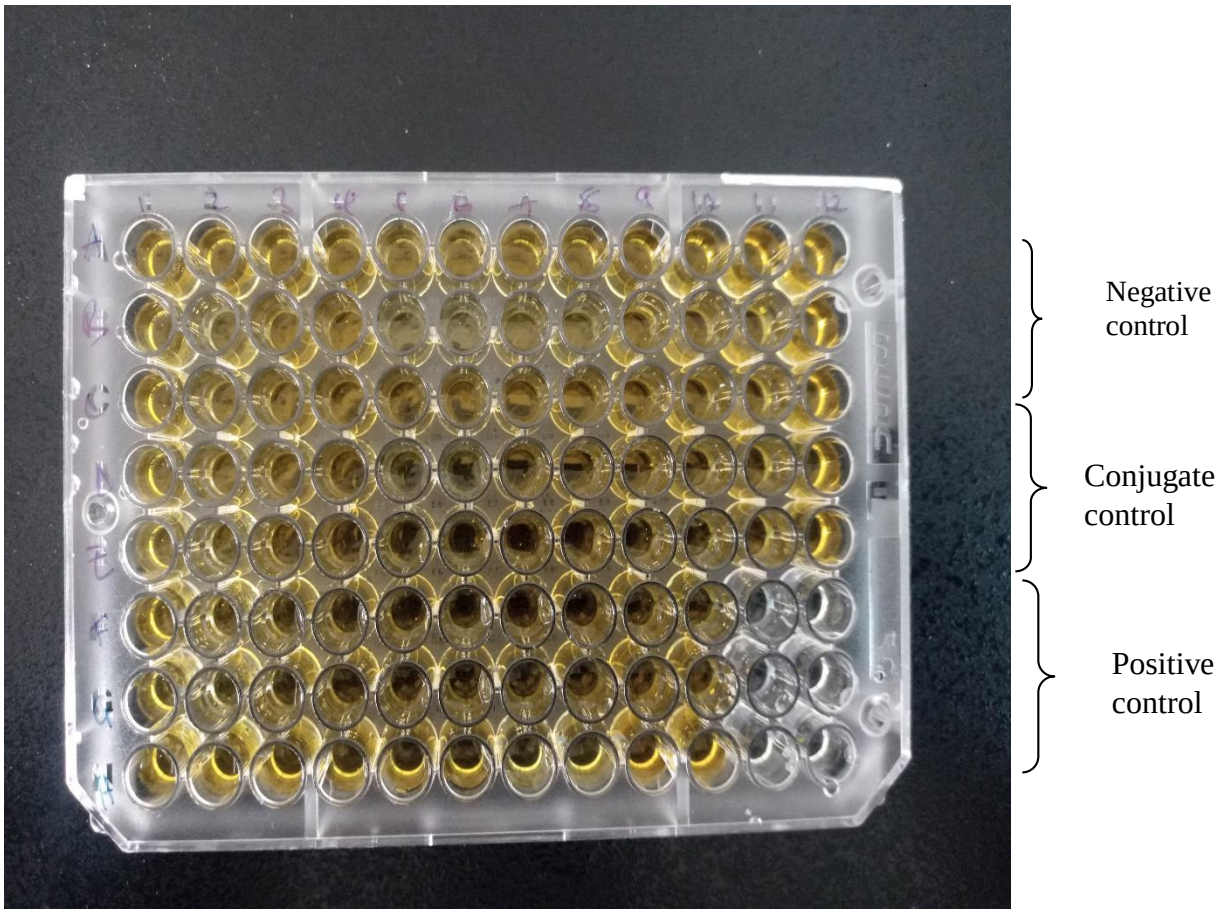

Figure 3: Microtitre Plate view

3.5.5 Data storage and analysis

R statistical software version 4.2.1 (2022-06-23 ucrt) was used to analyse the data. The prevalence of brucellosis was determined using descriptive analysis based on the cELISA tests. The chi-square was deployed for comparison between two or more proportions to determine the degree of association and statistical differences. Furthermore, the odds ratio (OR) was applied to investigate the relationship between brucellosis risk variables and seropositivity. Identification of risk variables linked with brucellosis spread in smallholder dairy cattle was done by using univariate and multivariate analyses (logistic regression).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 The sociodemographic profile of the respondents

The sociodemographic characteristics of the participants shows that 72% were males and 81% were aged between 41 and 60 years (Table 2).

Table 2: Sociodemographic characteristics of respondents participated in the study

Variables	Category	Meru	Hai	Total (%)
Gender	male	67	77	144 (72)
	Female	33	23	56 (28)
Age	18-25	1	0	1 (0.5)
	26-40	6	8	14 (7)
	41-60	79	83	162 (81)
	>60	13	11	24 (12)
Marital status	Single	11	9	20 (10)
	Married	88	83	171 (85.5)
	Divorced	1	0	1 (0.5)
	Widowed	3	5	8 (4)

4.1.2 General awareness and management practices of smallholder dairy cattle keepers on brucellosis

The respondent revealed different levels of general awareness and management practices on brucellosis in the study areas as summarized below (Table 5).

4.1.3 Respondents' general awareness on brucellosis

Although 74.5% of the participants knew the disease name, majority (87.9%) didn't know the clinical signs in animals. Among the clinical signs of the disease, only abortion was mentioned by 12.08% of the respondents (Fig. 4).

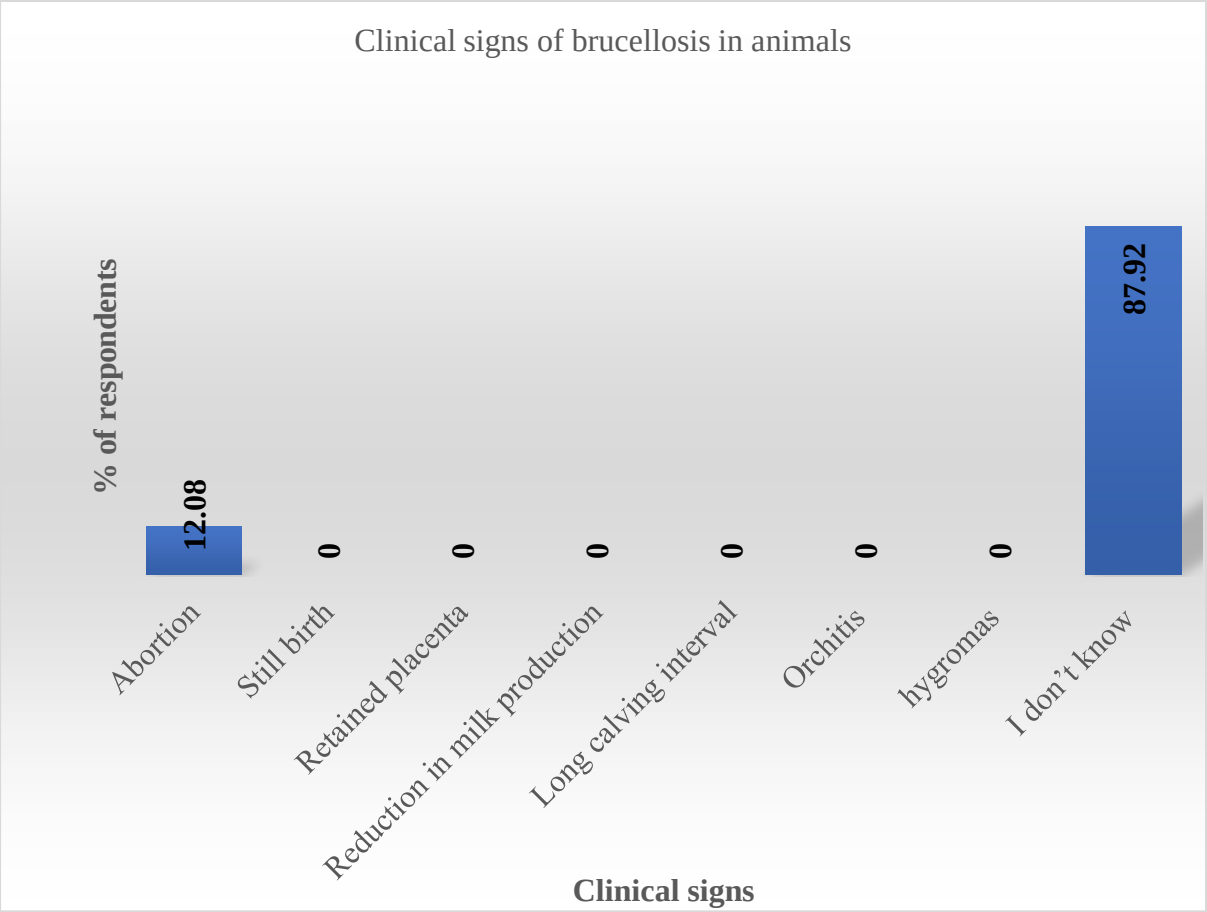

Figure 4: General awareness of the respondents on the clinical signs of brucellosis in the study areas

The results also showed that none of the respondents neither heard nor used brucellosis vaccine (S19) in the study areas whereas anthrax vaccine was well known by majority of respondents (Fig. 5).

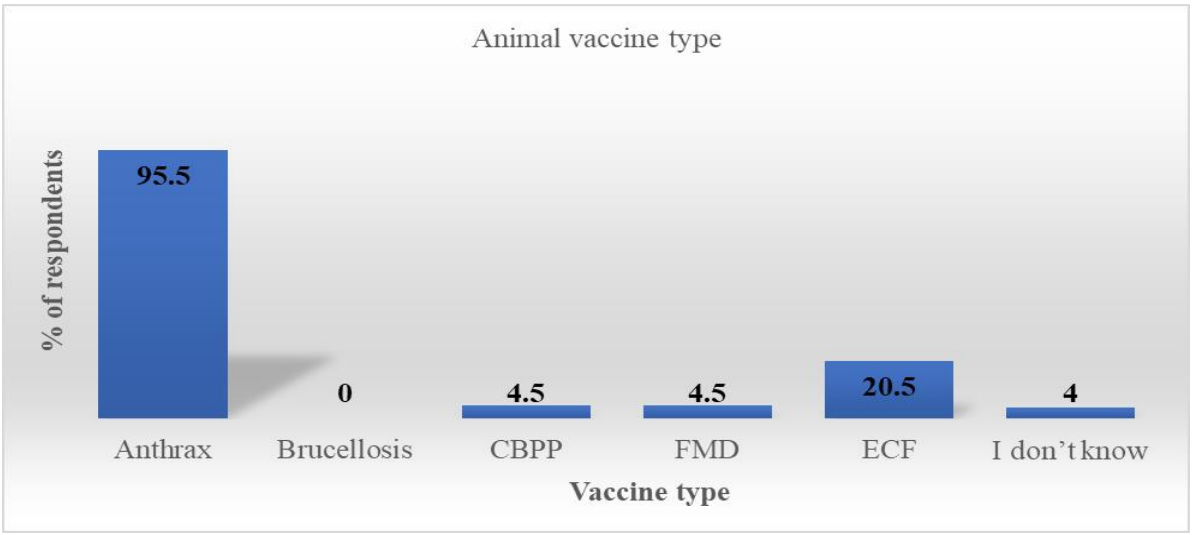

Figure 5: Respondents' awareness on livestock vaccines

4.1.4 Disposal methods of the aborted foetus and retained placenta

The results show that 7.5% of respondents' herd had a history of abortion while 1.5% reported retained placenta cases. Buring of both aborted foetus and retained placenta was the major disposal method in the study area (Fig. 6).

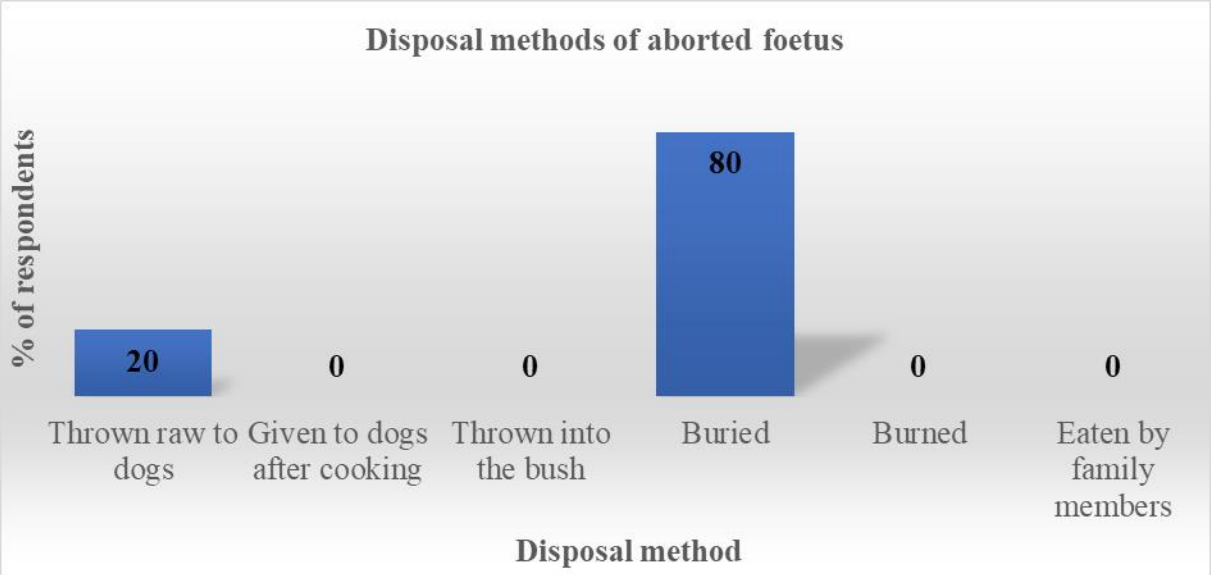

Figure 6: Disposal methods of the aborted foetus and retained placenta

4.1.5 Animal breeding methods used in the study areas

Most of the participants in the survey use artificial insemination compared to 32% who use natural mating (Fig. 7). However, 64% of participants acquired the bulls from other smallholder dairy cattle farms. The choice of the breeding method was based on cost (51.1%) as well as accessibility (34%).

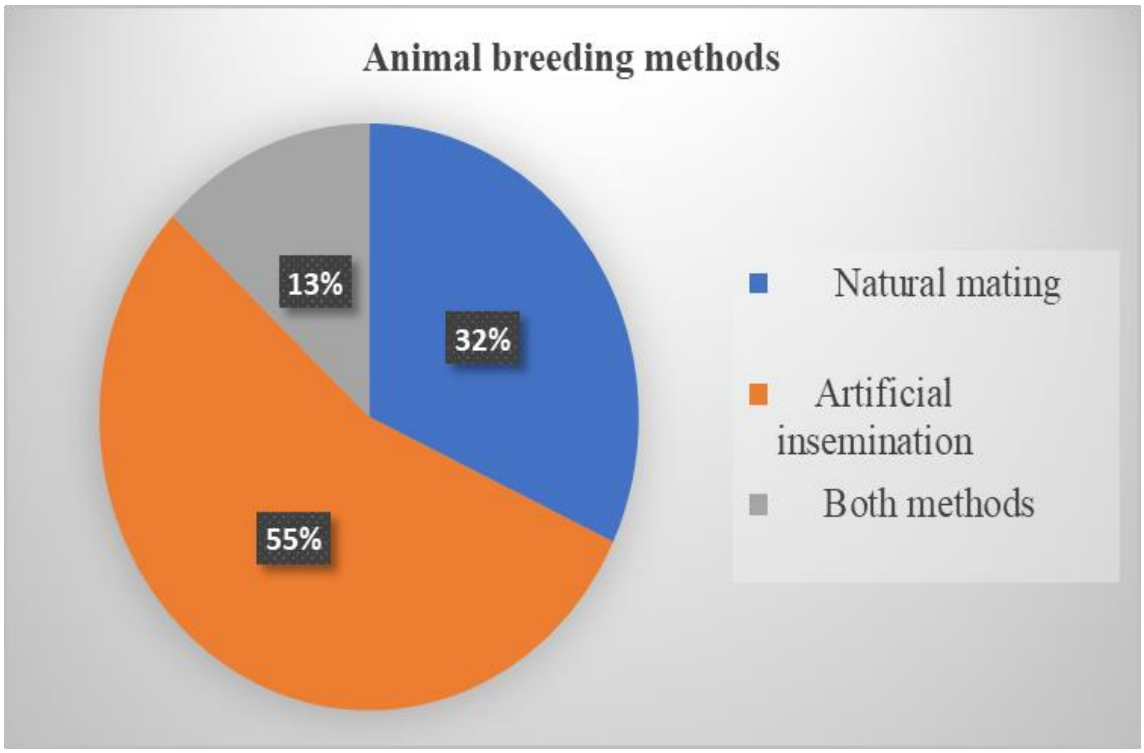

Figure 7: Service type used by smallholder dairy cattle farmers

4.1.6 Grazing system and animal interactions

Majority of the respondents practise zero grazing system compared to free-range system (Fig. 8). Furthermore, 99% of the respondents reported that their cattle were neither herded nor fed together with sheep and goats.

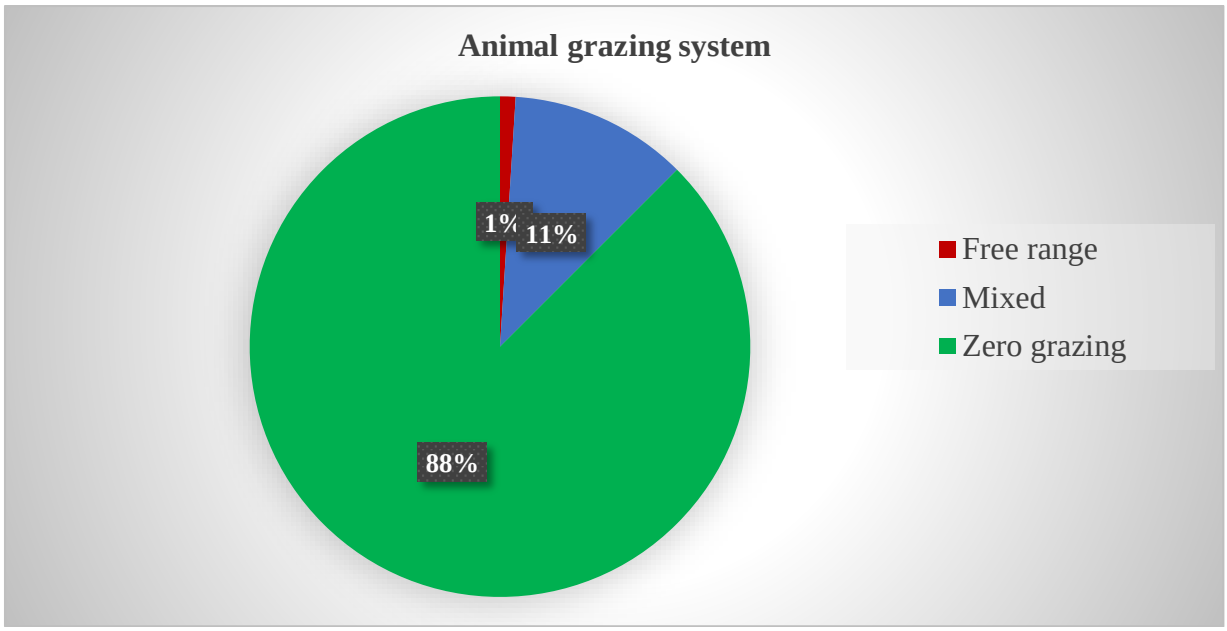

Figure 8: Smallholder dairy farms - grazing system in the study areas

4.1.7 Animals sources for heifer replacement

The results show that majority of the respondents acquired heifers from other smallholder dairy cattle farms within the District to replace the culled cows. In addition to that, the finding from this study shows that some smallholder cattle farmers upgraded female calves from their own farm to their breeding stock (Fig. 9).

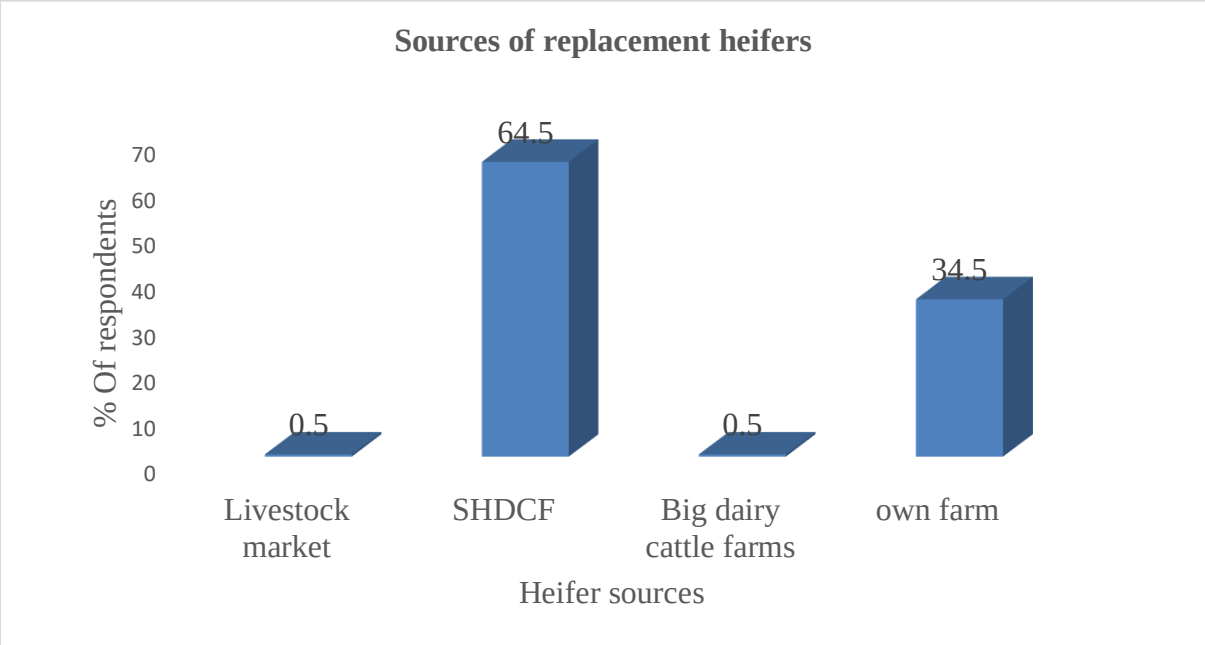

Figure 9: Source of heifer for replacement in the study areas

4.1.8 Vaccination against brucellosis

None of the respondents' herd received brucellosis vaccine for the past five years from 2018 to 2022. Inadequate understanding about the presence of brucellosis vaccine (84%), inadequate veterinary services (11%) and low mortality rate of brucellosis (5%) were the reasons to why smallholder dairy cattle farmers didn't vaccinate their cattle against brucellosis.

4.1.9 Management of animals that had failure to conceive

Although majority of farmers (90.5%) reported high conception rate by using both artificial insemination and natural mating; few farmers whose animals failed to conceive sold their cow to other smallholder farmers (Fig. 10).

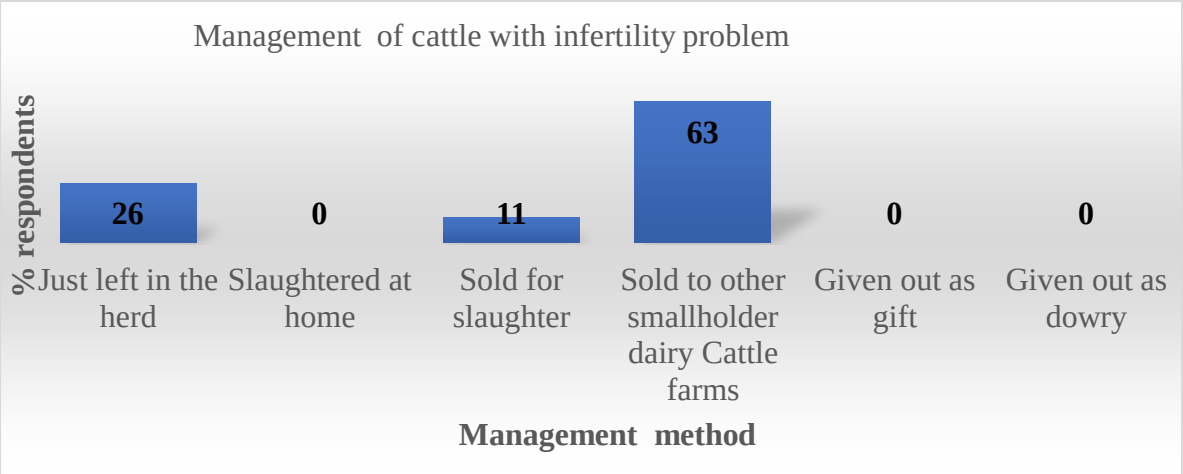

Figure 10: Handling of cattle that failed to conceive

4.1.10 Seroprevalence of bovine brucellosis in smallholder dairy cattle in Hai and Meru districts

A total of 400 dairy cattle from smallholder farms were tested for brucellosis with RBPT and cELISA tests. The animal-level seroprevalence of bovine brucellosis in Meru and Hai Districts was 0% and 0.5%, respectively, while the herd-level seroprevalence was 0% and 1% in Meru and Hai Districts, respectively. However, the seropositivity difference between Hai and Meru districts was not statistically significant at $p<0.05$ and 95% CI. The overall animal-level and herd-level seroprevalence of bovine brucellosis in the study areas was 0.25% and 0.5%, respectively (Table 3).

Table 3: The individual and overall seroprevalence of bovine brucellosis in Hai and Meru District Councils *(Results of c-ELISA across study Districts)*

Study District	Animal level seroprevalence		Herd level seroprevalence	
	N (n)	Prevalence (%)	N (n)	Prevalence (%)
Meru	200 (0)	0	100(0)	0
Hai	200 (1)	0.5	100(1)	1
Overall results	400 (1)	0.25	200(1)	0.5

N =Number of animals tested n = Number of positive reactors

4.1.11 Association of the seroprevalence of bovine brucellosis across host-related risk factors

Analysis of animal level risk variables and seropositivity of brucellosis among smallholder dairy cattle showed that the animal that tested positive was an adult Friesian female with neither abortion history nor infertility problems as well as history of retained placenta. Also, the positive reactor animal had no history of retained placenta. All animal levels risk factors (Age,

sex, breed, abortion history, retain placenta and failure to conceive) were statistically insignificant (p values > 0.05) under univariable analysis (Table 4).

Table 4: Association of the seroprevalence of bovine brucellosis across host-related risk factors

Risk factor	Category	N (+)	Prevalence (%)	χ^2	p value
Age	Young	22(0)	0		
	Adult	378(1)	0.25	1.51e-28	1
Sex	Male	23(0)	0		
	Female	377(1)	0.25	9.95e-32	1
Breed	Jersey	33(0)	0		
	Ayrshire	61(0)	0		
	Friesian	306(1)	0.25	0.30369	0.8591
History of abortion	Yes	29(0)	0		
	No	371(1)	0.25	1.72e-29	1
Failure to conceive	Yes	30(0)	0		
	No	370(1)	0.25	1.86e-27	1
History of RP	Yes	7(0)	0		
	No	393(1)	0.25	1.26e-26	1

N =Number of animals tested; n = Number of positive reactors; RP=Retained placenta

4.1.12 Information about the positive reactor animal

Further information revealed that the seropositive cattle was a Friesian adult female kept under zero grazing system. The herd had 4 dairy animals with no small ruminants. Natural mating and artificial insemination were interchangeably used to service the animals in the herd. The knowledge and practises related to bovine brucellosis and husbandry with the herd owner did not differ from the rest of farmers.

4.1.13 Association of the seroprevalence of bovine brucellosis and the knowledge, management and practice related risk factors (Herd level variables)

All herd level variables (grazing system, type of service, source of heifers, contacts and vaccination) were not associated with the brucellosis seropositivity (p values >0.05 (Table 5).

Table 5: Influence of smallholder farmers knowledge, altitude and management practices on seroprevalence of bovine brucellosis

Risk factor	Category	N (+)	Prevalence (%)	χ^2	p value
Grazing system	Free range	4(0)	0	0.14322	0.9309
	mixed	46(0)	0		
	zero grazing	350(1)	0.25		
Type of service	AI	224(0)	0	2.155	0.3404
	Bull	126(0)	0		
	Both	50(1)	0.25		
Heifer source	livestock markets	2(0)	0	0.01861	0.8915
	smallholder dairy cattle farms	258(0)	0		
	Big dairy cattle farms	2(0)	0		
	own farm	138(1)	0.25		
Frequent contacts with other herds	Yes	2(0)	0	8.24e-23	1
	No	398(1)	0.25		
Disease name	Yes	299(1)	0.25	0	1
	No	101(0)	0		
Brucellosis diagnosis	Yes	11(0)	0	2.56e-26	1
	No	389(1)	0.25		
Vaccinated against brucellosis	Yes	0(0)	0	8.38e-27	1
	No	400(1)	0.25		
Access to veterinary services	Yes	(0)	0	1.26e-26	1
	No	44(1)	0.25		
Livestock sharing house at night	Yes	1(0)	0	8.38e-27	1
	No	399(1)	0.25		

N =Number of animals tested; n = Number of positive reactors

4.2 Discussion

This research was carried to determine the current status of brucellosis and possible risk factors in the smallholder dairy cattle in Hai and Meru Districts.

The findings from this study indicate no positive cattle sampled from Meru district, however, one dairy cattle in Hai district indicated to have been exposed to brucella infection. This indicates that the two districts are not among the brucellosis hotspots as informed by other investigators (Mengele *et al.,* 2023; Shirima *et al.,* 2018).

Similarly, in this study, it was found that dairy farmers are not using the S19 vaccine against brucellosis and this indicates that the infected animal was due to natural exposure of the pathogen. The low brucellosis seroprevalence in Hai and Meru is within the range of brucellosis

studies on dairy cattle that were conducted in several regions of Tanzania (Mengele *et al.,* 2023; Mdegela *et al.,* 2004; Karimuribo *et al.,* 2007; Alexander, 2017; Mhozya, 2017). Similarly, the results of this investigation concurs with other research findings across the World (Kothowa *et al.,* 2021; Hesterberg *et al.,* 2008; Getahun, 2021; Nguna *et al.,* 2019; Hassan *et al.,* 2014). The results of this study are contrary to the findings of other studies where the seroprevalence was high (Swai & Schoonman, 2010; Mengele *et al.,* 2023). The difference in seroprevalence in various studies in Tanzania could be caused by variations in study design, farming systems, management practices and other biosecurity measures taken by farmers. The very low seroprevalence obtained in this study might be attributed by a number of factors such as zero grazing system, animal replacement practices, disposal method of the aborted foetus and breeding methods.

In this study, it was found that zero grazing system was the leading grazing system practiced by most of the smallholder dairy cattle farmers. Through zero grazing system, animals are fed on fodder using cut and carry practices. Therefore, there is less interactions of animals between herds thus acts as one of the key biosecurity control options against disease transmission including brucellosis. This observation coincides with the results of other investigators (Karimuribo, 2007; Swai & Schoonman, 2010) that zero grazing system minimize the level of infection since animals from different herds do not interact to each other. This is in contrary to other farming systems such as pastoral and agro-pastoral systems where herds with multispecies interact frequently in grazing and watering points thus perpetuates disease transmission (Assenga *et al.,* 2015; Shirima, 2005). Furthermore, the zero grazing system practised in the study areas separates small ruminants from being herded together with the dairy cattle thus minimize the cross-infection risk as well (Shirima, 2005; Rubegwa, 2015; Oromia *et al.,* 2022; Mengele *et al.,* 2023).

Based on the fact that both districts practised dairy farming for decades, animal replacement becomes feasible from within the farms/ districts minimising introduction of animals from outside herds/districts. The mode of animal acquisition in the study areas does not favour the introduction of animals that might be infected from other areas. This observation concurs with the findings from other studies (Shirima, 2005; Karimuribo, 2007; Alexander, 2017). However, the findings of this study are different from that of Rubegwa *et al.* (2015) and Abera *et al.* (2019) who reported higher bovine seroprevalence in homebred animals than brought animals.

Artificial insemination could have contributed to the observed low seroprevalence of brucellosis in the sampled cattle. The disease's free semen used in artificial insemination limits possible transmission of Brucella pathogens from the infected bulls to cows. This findings from the study areas is also supported by others elsewhere (Corbel, 2006; Shirima, 2005) and Mfune (2015) who reported that there is high prevalence of Brucella infection in areas with low use of artificial insemination breeding method. Although proportion of dairy cattle farmers used bulls for mating; they are sourced from within the district and when screened were negative to both RBPT and cELISA. The use of bulls is common in pastoral farming systems thus may attribute to the level of infection reported compared to dairy cattle farming (Mellau & Wambura, 2009; Swai & Schoonman, 2010; Nguna *et al.*, 2019).

Reports of abortion incidences and retain placentas in the current study may be clear evidence of low reproductive diseases including brucellosis. From this study, it was found that abortion and retained placenta were not common cases to happen in dairy cattle. However, cases of aborted foetus and retained placenta were disposed of properly by burying them onto the ground. This further prevents disease perpetuation as reported earlier that feeding raw to dogs amplify spread of the disease (Shirima, 2005; Sijapenda *et al.*, 2017; Ntirandekura *et al.*, 2018; Ismail *et al.*, 2019; Ntivuguruzwa *et al.*, 2020; Mengele *et al.*, 2023).

Both animal and herd level risk factors investigated in this study were not associated with the obtained low seroprevalence. The investigated animal levels risk factors were such as sex, age, breed type, abortion history and retained placenta cases and herd level risk factors included farmer's awareness of the disease name, clinical signs of brucellosis in cattle, proper disposal methods of the aborted fetus, management of infertile animals, grazing system, breeding method, animal replacement, animal housing system, animal vaccination. It was not possible to compute the association between the investigated risk factors and the obtained low seroprevalences.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the findings of this study, it can be concluded that:

(i) Brucellosis seroprevalence in smallholder dairy cattle in Hai and Meru districts is relatively low.

(ii) Zero grazing system coupled with in-house breeding and animal replacement from within the study areas may have provided biosecurity measures for brucellosis spread.

(iii) In light of the brucellosis control program in the study areas, this study highlights the lack of brucellosis vaccination coverage among smallholder dairy farmers.

(iv) This study highlights low understanding of smallholder farmers on control of brucellosis through managerial practices.

5.2 Recommendations

From the findings of this study, the following recommendations are made:

(i) There should be education campaign on brucellosis for smallholder dairy cattle farmers to increase their awareness of the disease.

(ii) Brucellosis certification scheme initiated after this study be strengthened and monitored to ensure a disease-free area.

(iii) In the event of low seroprevalence, the surveillance monitoring approach may shift to bulk milk sampling to detect exposed herds.

(iv) The smallholder dairy cattle farmers should keep on conducting proper management and practices that minimize the risk of brucellosis transmission such as zero grazing system, acquiring animals from brucellosis free herds, breeding through artificial insemination, burying of aborted fetus and retained placenta, test and slaughter of exposed animals. separation of cattle house from that of sheep and goat.

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APPENDICES

Appendix 1: Consent Form

PART I: INFORMATION SHEET

Study title: ***Epidemiology of brucellosis among smallholder dairy cattle in Hai and Meru District Councils Northern Tanzania.***

Researcher details: My name is Peter Jiday Elisha, a Masters student at Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania.

Aim of the study

The current study seeks to establish the current magnitude of brucellosis and its associated risk factors among smallholder dairy cattle in Hai and Meru District Councils for proper control and prevention approaches to encounter social-economic impacts of the disease.

Participation in this study

You are asked to respond to questions about the potential major risk variables during this investigation. For cattle brucellosis among smallholder dairy Cattle in Hai and Meru District Councils Northern Tanzania. The session is designed to take place not more than half an hour. It is not a command to share personal views, practices, or experiences concerning a specific question. Also, you are under no responsibility to submit any information with which you are uncomfortable.

Risks

I do not anticipate any risks occurrence during the study.

Benefits

There is no direct benefit which was obtained from this study but better understanding of current magnitude of brucellosis and its associated risk variables among smallholder dairy Cattle in Hai and Meru District Councils for proper control and prevention approaches to encounter social-economic impacts of the disease.

Confidentiality

To protect each participants' privacy, all data collected from the study was coded. No names or personal details was revealed while discussing or communicating results. All files and data obtained by the investigators was secured. And discarded when it has been thoroughly analyzed.

Costs/Compensation

Participant will not be required to make any payments to take part in the study or receive any compensation for agreeing to be part in the study.

For further information, questions or any queries, you can contact:

Mr. Peter Jiday Elisha (Student researcher)

Mobile number: +255759919463/0712264540, Email: *elishap@nm-aist.ac.tz*

NM-AIST, Tanzania

PART II: CERTIFICATE OF CONSENT

Statement of the consent

I have read the information given/or has been read to me. I had the chance to ask questions regarding it, and all of them have been answered to my satisfaction. I actively accept to participate in this study.

Name of participant: ……..….

Signature of participant: ……

Date……..……

Statement by researcher/ a person taking consent

I confirm that the participant was given the opportunity to ask questions concerning the study, and that I correctly answered all of the participant's questions to the best of my ability. I declare that the consent was given freely and voluntarily, and that the individual was not pushed into giving consent.

Name of researcher/a person taking consent: ……

Signature of researcher/a person taking consent: ……..……

Date: ……

Appendix 2: Data collection questionnaire

Cross-Sectional Questionnaire Survey on Risk Factors for Brucellosis in Smallholder Dairy Cattle in Hai and Meru District Councils Northern Tanzania

1.0 BACKGROUND DATA

Interview's date (dd/mm/yy) ....., ...

Interviewees names ..... Sex ..... Age .....

Head of household ..... Sex ..... Age .....

Marital status ....

Village ..... Ward ..... Division..... District Council.....

2.0 GEOGRAPHIC INFORMATION

2.1 GPS coordinates ..... S ..... E Altitude(m) .....

2.3 Distance to nearest neighbour (meters/km) .....

3.0 ANIMAL MOVEMENTS AND CONTACTS

3.1 How many animals do you have? (Give exact figure or range).

Animals	Interviewee's response		Direct observation	
	Females	Males	Females	Males
Cattle				
Goats				
Sheep				
Donkeys				
Pigs				
Calves				

3.2 Where do you acquire heifers?

a) Livestock Markets

b) Smallholder dairy Cattle { }

c) Big dairy Cattle

d) Own farm

e) Other……..

3.3 What type of grazing system do you apply?

a) Zero grazing

b) Free-range { }

c) Mixed

If the answer is ‘’b’’ or ‘’c’’ then, continue to the next question, if is ‘’a’’ skip this question.

Contact with other cattle (l=often, 2=occasionally, 3=never)

HERDS	**DRY SEASON**		**WET SEASON**	
	Grazing areass	Watering points	Grazing areass	Watering points
Cattle from other herds				

3.4 Are the cattle herded with sheep and goats?

During the dry season (Yes/No) ..... During the wet season (Yes/No) .....

3.5 Do your cattle stay with other livestock during the night? (Y*es/N*0) .....

4.0 HERD MANAGEMENT

4.1 What breeding techniques do you employ?

a) Natural mating

b) Artificial insemination { }

c) Both methods are used interchangeably

4.2 Why that breeding method was used?

a) Less expensive

b) Easily accessible { }

c) Very efficient

d) Others

If the answer to 4.1 is ‘’a’’ or ‘’c ‘’,go to 4.3, if the answer is ‘’b’’ skip 4.3

4.3 Where do you acquire the bull?

a) Own farm
b) Small holder dairy Cattle { }
c) Big dairy Cattle
d) Others

4.3 How many calves were born in the past two years in this herd? Give the exact figure…….

4.4 Do you own a cow that gave birth prior to 2020 but has since been unable to conceive? (Yes/No) …….

If Yes, what did you do with such animals?

	Yes/No
Just left in the herd	
Slaughtered at home	
Sold for slaughter	
Sold to other smallholder dairy Cattle farms	
Given out as gift	
Given out as dowry	
Others (specify)	

4.5 Did any of your cow abort between the Years 2018-2020? (yes/No) ..... .

If yes indicate how many and the stage of abortion:

Animal/Stage	Early	Mid	Late	Unknown
Cow				

4. 6 Which techniques did you employ for the aborted fetus? (Write V when the answer is Yes and X when is No).

Where	Yes/NO
Thrown raw to dogs	
Given to dogs after cooking	
Thrown into the bush	
Buried	

Burned	
Eaten by family members	
Others (specify)	

4.7 What was the trend of milk production before and after abortion? Please specify the quantity variation…….

4.8.1 Have there been any instances of retained placenta between 2018 and 2020? (Yes/No) …….

If so, how many cases in the flock have retained placentas?4.8.2 Which method was used to dispose the retained placenta?

Where	**Yes/NO**
Thrown raw to dogs	
Given to dogs after cooking	
Thrown into the bush	
Buried	
Burned	
Eaten by family members	
Others (specify)	

4.9. What were the cost of treating the retained placenta? Write the exact figure…….

5. BRUCELLOSIS HISTORY, KNOWLEDGE AND ACCESS TO BRUCELLOSIS VACCINES

5.1 Have you ever heard of the disease known as brucellosis?? (Yes/No) **…….**

If no to 5.1, skip to 5.9

5.2 Can you tell us what clinical signs of brucellosis in cattle are?

Ask the respondent if they believe each of the clinical symptoms listed is connected to brucellosis as you go through the list of symptoms. After prompting, write down a Yes (Y), No (N), or Don't Know (DK) response. Add any other symptoms or indications that have been reported in the text box.

I don't know	…….
Abortion	(Y es/No) **…….**
Still birth	(Y es/No) **…….**

Retained placenta	(Y es/No) **…….**
Chronic reduction in milk production	(Y es/No) **…….**
Long calving interval	(Y es/No) **…….**
Orchitis (In male)	(Y es/No) **…….**
hygromas (In male)	(Y es/No) **…….**
Any other (Specify)	(Y es/No) **…….**

5.3 Have your cattle been diagnosed with brucellosis? (Yes/No) **…….**

If no to 5.3, skip to 5.6

5.4 When was the diagnosis made?

Month Year

5.5 Where was the diagnosis made?

5.6 Why your cattle were not diagnosed with brucellosis?

Ask the respondent if they believe any of the list of reasons why cattle were not diagnosed with brucellosis is related to the disease. After prompting, write down a Yes (Y), No (N), or Don't Know (DK) response. jot down any extra information in the text box that is reported.

I don't know	…….
Expensive	(Y es/No) …….

Lack of access to veterinary services	(Y es/No) …….
Lack of livestock officer in the areas	(Y es/No) …….
Any other (Specify)	

5.7 Had you heard of a livestock vaccine (Yes/No) **…….**

Go through the list of livestock vaccines and prompt the respondent to find out vaccine awareness Record a Yes (Y), No (N) Don't Know (DK) response after prompting.

Vaccine	Response
Anthrax	(Y es/No) **…….**
Brucellosis	(Y es/No) **…….**
CBPP	(Y es/No) **…….**
FMD	(Y es/No) **…….**
ECF	…….
I don't know	
Any other (Specify)	

5.8 Do your cattle have brucellosis vaccinations? (Y es/No) **…….**

If Yes to 5.8, Ignore 5.9

5.9 Why your cattle were not vaccinated against brucellosis?

Examine the list of reasons why cattle were not immunized against brucellosis and ask the respondent if they believe each is related to the disease. After prompting, write down a Yes (Y), No (N), or Don't Know (DK) response. jot down any extra information in the text box that is reported.

I don't know	…….

Expensive	(Y es/No) **…….**
Lack of access to veterinary services	(Y es/No) **…….**
Any other (Specify)	(Y es/No) **…….**

6. MILK DISTRIBUTION CHANNELS, PRICES AND VALUE CHAIN

6.1What is the quantity of milk production per farm per day? (Use average per cow per day to attain the total milk production)

6.2 How do you distribute the milk from your farm?

a) left at home

b) Sold to Milk venders { }

c) Sold to Milk Collection Centres

d) Sold to both milk Vendors and collection centres

If the answer is “b”, skip 6.4 and 6.5, if the answer is “c” then skip 6.3

6.3 What is the quantity of milk sold to milk venders per day? Write the exact figure….

6.4 What is the quantity of milk sold to milk collection centres? Write the exact figure….

6.5 Mention the Milk collection centre where you sell milk from your farm…….

6.6 Mention out the milk price at:

	MILK PRICES	
	At milk vendors	At Milk Collection Centre
	TZS……	TZS……

RESEARCH OUTPUTS

(i) Publication

Elisha, P. J., Kimaro, E. G., & Shirima, G. M. (2023). Seroprevalence of bovine brucellosis and associated risk factors among smallholder dairy cattle farmers in Hai and Meru District Councils Northern Tanzania. *SVU-International Journal of Veterinary Sciences*, 6(4), 38-54.

(ii) Poster Presentation