Thesis ref. No._____

SERO-PREVALENCE AND RISK FACTORS ASSOCIATED WITH BRUCELLOSIS IN RUMINANTS AND HUMANS IN SELECTED DISTRICTS OF WEST SHOA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF CLINICAL STUDIES

MASTERS PROGRAM IN VETERINARY EPIDEMIOLOGY

By:

Wakuma Mitiku Bune

JUNE, 2021

BISHOFTU, ETHIOPIA

SERO-PREVALENCE AND RISK FACTORS ASSOCIATED WITH BRUCELLOSIS IN RUMINANTS AND HUMANS IN SELECTED DISTRICTS OF WEST SHOA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Epidemiology.

By:

Wakuma Mitiku Bune

JUNE, 2021

BISHOFTU, ETHIOPIA

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As MVSc research advisers, we here certify that we have read and evaluated the Thesis prepared under my guidance by **Wakuma Mitiku entitled: "Sero-prevalence and risk factors associated with brucellosis in ruminants and humans in selected districts of West Shoa zone, Oromia regional state, Ethiopia**"; we recommend that it can be submitted as fulfilling the MVSc thesis requirement.

Dr. Teshale Sori (DVM, MSc, PhD, Asso. Prof.)		17/06/2021
Major Advisor	Signature	Date
Dr. Getachew Tuli (DVM, MSc)		17/06/2021
Co- Advisor	Signature	Date
Mr. Teferi Benti		17/06/2021
Co- Advisor		
Submitted by: Wakuma Mitiku	_____	_____
Name of Student	Signature	Date
Dr. Haileleul Negussie (DVM, MSc, PhD, Assoc. prof.)	_____	_____
Department chair person	Signature	Date

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As members of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Wakuma Mitiku** Titled: "**Sero-prevalence and risk factors associated with brucellosis in ruminants and humans in selected districts of West Shoa zone, Oromia regional state, Ethiopia**". We recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Epidemiology

_____	_____	_____
Chairman	Signature	Date
_____	_____	_____
External Examiner	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
Dr. Haileleul Negussie		
	_____	_____
Department chair person	Signature	Date

STATEMENT OF THE AUTHOR

I, the undersigned, declare that the thesis is my original work and that all sources of material used in this thesis have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for the MVSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture, and has been deposited at the University/College library to be made available to borrowers in accordance with the rules of the library. I sincerely declare that this thesis is not being submitted to any other institution for the award of any academic degree, diploma, or certificate anywhere in the world.

Brief quotations from this thesis are permissible without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduce this manuscript in whole or in part may be granted by the major advisor or head of the major department, or the Dean of the College, if the proposed use of the material is in the interests of scholarship in his or her judgment. In all other cases, however, permission from the author is required.

Name: Wakuma Mitiku Bune Signature: _____
College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia
Date of Submission: 17/06/2021 GC

ACKNOWLEDGEMENTS

Above all, thanks to my almighty God for his help in giving me the courage to cop up complicated situations I faced for pursuing my study and for his help and courage during my whole study time.

The author expresses his deepest and sincere gratitude to academic advisor Dr. Teshale Sori, Addis Ababa University (AAU), college of veterinary medicine and agriculture (CVMA), for his work supervision, sound advice, time devotion to correct this manuscript and professional guidance at all stage of the work.

The author is also adding special thanks to Dr. Getachew Tuli, Mr. Teferi Benti, and Mr. Bayata Senbeta, Sebata National Animal Health Diagnosis and Investigation Center under the Ministry of Livestock and Fish resource for their laboratory reagent and material support and the author wishes to extend his gratitude to Dr. Adugna Woyessa and Dr. Fasil Mengistu, Ethiopian public health institute (EPHI), for their encouragement and positive cooperation during his research work.

The author is grateful to the Ambo University for providing him study leave and guarantees his salary during the study time and Addis Ababa University, College of Veterinary Medicine and Agriculture (AAU, CVMA), Bishoftu, Ethiopia, academic and support staff members of the college, for their positive cooperation during the research work.

The author particularly thankful to the livestock and fishery resource development office staffs in Bako, Nono and Ilu Galan districts for their support and collaboration during the study period and also want to convey his heart felt gratitude to the livestock owners for answering the survey questions honestly and allowing their animals for sample collection.

The author wishes to express his deepest love and gratitude to his spouse Abonesh Bedasa, for her incessant love, morals, and care of his life and Kid Melba Wakuma next to God and encouragement through his ups and down in personal and social life.

TABLE OF CONTENTS

CONTENTS **PAGES**

TABLE OF CONTENTS (Continued)

LIST OF ABBREVIATIONS

AMOS	Abortus-Melitensis-Ovis-Suis
BPAT	Buffered Antigen Plate Agglutination
C-ELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation Test
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
DS	Doxycycline with Streptomycin
ELISA	Enzyme Linked Immunosorbent Assay
HRP	Horseradish Peroxidase
iELISA	Indirect Enzyme Linked Immunosorbent Assay
IS711	Insertion Sequence 711
LPS	Lipopolysaccharide
mAb	Monoclonal Antibody
OD	Optical Density
OIE	Office International Des Epizooties
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
RFM	Retained Fetal Membrane
ROC	Receiver Operating Curve
RT-PCR	Real Time-PCR

LIST OF FIGURES

PAGE

LIST OF TABLES

PAGE

LIST OF ANNEXES

PAGE

ABSTRACT

A cross-sectional seroprevalence study was conducted in Bako Tibe, Ilu Galan, and Nono districts of the West Shoa zone, from January - May 2021 to estimate the seroprevalence and identify associated risk factors of brucellosis in ruminants and humans, as well as to assess the knowledge and practices of livestock owners towards the disease. A total of 295 blood samples were collected for serological tests from ruminants using a simple random sampling, whereas the districts and kebeles were selected purposively. In addition, 102 human sera were included in the study. Rose Bengal Plate test was used to screen the serum samples, and indirect enzyme-linked immunosorbent assay was employed as a confirming test. Besides, information was collected on the individual animal and herd-level risk factors using a structured questionnaire. Descriptive statistics were used to summarize the prevalence of brucellosis and present the questionnaire survey results. Firth's bias-reduced logistic regression was used to determine the association between the prevalence of brucellosis and the risk factors. The overall seroprevalence of brucellosis was 3.3 % (95 % CI: 1.73-6.34) by Rose Bengal Plate test and 1.3 % (95 % CI: 0.43 -3.67) by indirect enzyme-linked immunosorbent test, of these,1.17% (95 % CI: 0.14 - 4.18) in cattle, 1.63% (95 % CI: 0.04 - 8.7) in sheep and 1.56% (95 % CI: 0.03 - 8.4) in goats. The result of univariable firth's bias-reduced logistic regression analysis indicates that animals with the history of abortion (OR= 26, P= 0.003), species composition (OR= 8, P= 0.023), and retained fetal membrane (OR=9, P=0.034) were found significantly associated with brucellosis. Nevertheless, in the multivariable firth's bias-reduced logistic regression analysis, history of abortion (OR=10.72, 95 % CI: 1.06 -131.26, P = 0.044) and species composition (OR=12.37, 95 % CI:0.98 -155.67, P = 0.03) were statistically significant risk factors of ruminant brucellosis. Four blood clots from seropositive animals were further tested with real-time PCR and the result revealed that all samples were negative for *IS711* primers. Similarly, no human sera were found positive for *Brucella* antibodies. A total of 120 peoples were questioned to evaluate their awareness and practices regarding to brucellosis. Accordingly, most respondents 71.7% did not know brucellosis and 50% of them practices handling animals' delivery. In conclusion, despite the low figure of brucellosis, the free movement of animals across herds could make it a source of infection

for other herds. The study also shows that, despite having some understanding about zoonosis, the community's practices are poor. As a result, seropositivity in animals may suggest that brucellosis poses a public health risk. This necessitates a more detailed epidemiological and genomic assessment to identify the specific *Brucella* species found in the area's animals and humans.

Key words: *Brucellosis, Ruminants, Risk factors, Seroprevalence, West Shoa, Ethiopia*

1. INTRODUCTION

Brucellosis is one of the most important and widespread zoonotic diseases of domesticated animals and humans in the world (Zhou *et al*., 2020). It's an infectious bacterial disease caused by members of the genus *Brucella* and characterized as small, facultative, gram-negative, non-motile, non-spore-forming, and rod-shaped (coccobacillus) bacteria. Currently, there are 11 recognized *Brucella* species, and six of them, are known to be pathogenic for both animals and humans, namely: *B. abortus, B. canis, B. inopinata, B. melitensis, B. pinnipedialis,* and *B. suis.* However, *B. melitensis*, which mainly affects goats and sheep, causes serious infection and is responsible for the most of global morbidity (Hull and Schumaker, 2018).

Although brucellosis has been eradicated from most developed countries, its occurrence is increasing in developing countries (Tulu and Deresa, 2020). In low-income countries like Ethiopia, the disease is often under-reported and there is no effective control measures, making brucellosis a disease of major public health, economic, and livelihood burdens (Pieracci *et al*., 2016). Brucellosis is primarily a reproductive disease characterized by abortion in the last trimester and retained placenta in the female animals whereas orchitis and epididymitis with frequent sterility in males. Brucellosis can result in severe productivity loss in farm animals due to different factors like miscarriage, long calving to conception intervals and low milk production (Franc *et al*., 2018; Workalemahu *et al*., 2017), and can cause a chronic and febrile illness in humans (Franc *et al*., 2018).

Brucellosis is a major animal and public health problem in areas with intensive, mixed types of farming, and where owners co-habit with their animals during the night. In these systems, the mixed, migratory, and free-roaming nature of livestock makes it impractical to separate diseased animals from healthy ones exacerbating its transmission (Pal *et al*., 2017). In the livestock industry, the economic impact of brucellosis is mainly attributed to abortion which mostly occur during the last trimester. In some cases, abortions are followed by temporary or definitive infertilities also with a decrease or a total absence of milk

production (Radostits *et al*., 2007). Thus, brucellosis can have a considerable economic impact at the household and national level.

Humans may become infected by the brucellosis through break in the skin, following direct contact with infected tissue, aborted fetus, vaginal discharge, placenta, blood, and urine (Pal *et al*., 2017). Foodborne infection occurs following ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals (Lakew *et al*., 2019). Occupational airborne infection in laboratories and abattoirs have also been documented. Accidental inoculation of live vaccines, such as *B. abortus* Strain 19 and *B. melitensis* Rev.1, can also occur resulting in human infections (OIE, 2013).

Diagnosis of clinical Brucellosis is initially made by use of appropriate serological tests, and confirmed by bacteriological isolation and identification of the agent (OIE, 2013). Different serological diagnostic techniques are suitable for screening large herds which are easy to perform, cheap, and economical. Yet, false-positive reactions may be observed due to cross-reactions with non-specific antibodies (Ahmed *et al*., 2016). Rose Bengal plate agglutination test (RBPT) is an economical, rapid, and simple screening assay for brucellosis. Nevertheless, because of high sensitivity, serological cross-reactivity may occur which gives rise to false-positive reactions. That is why the existing OIE guidelines recommend that all positive RBPT results should be confirmed by quantitative assays like ELISA and CFT (OIE, 2013; Poester *et al*., 2013).

There is no documented evidence of how and when brucellosis was imported and established in Ethiopia. However, several serological surveys conducted over the previous three decades have revealed that it is endemic and prevalent (Bekele and Kasali, 1990; Asfaw *et al*., 1998). The disease is prevalent in domestic ruminants and camels in lowland areas of Ethiopia (Megersa *et al*., 2011b; Sintayehu *et al*., 2015). Also, a large number of studies on cattle and small ruminants have reported seroprevalence ranging from 0.0% to 11% (Kebede *et al*., 2008; Lakew *et al*., 2019) and 0.0 % to 9.7.% (Teshale *et al*., 2006; Tesfaye *et al*., 2020) respectively. Few serological studies on human brucellosis reported a prevalence as high as 34.9% (Genene *et al*., 2009). That is, the previous studies revealed that brucellosis is a public health concern in communities where people live in close contact with their animals. Brucellosis can easily transmitted through raw milk consumption, direct

contact with infected animals, a contaminated environment, and occupational exposure (OIE, 2013). Therefore, any plan for control of brucellosis should start by appraising its importance through developing a different epidemiological framework such as determining the disease's prevalence and examining the predisposing factors in a given area. Despite presence of large number of ruminant livestock sharing shelters with humans, no study has been undertaken in west Shoa zone. Besides, the districts selected for this study are lowland where number febrile diseases have been reported in humans. Most of these febrile diseases are considered as fever of unknown origin if they are negative for malaria. Therefore, this study was conducted with the following objectives.

- To estimate seroprevalence of brucellosis in cattle, sheep, and goats in selected districts of west Shoa Zone
- To identify the potential risk factors of brucellosis in cattle, sheep and goats
- To estimate the prevalence of brucellosis in human patients at Bako Tibe and Ilu Galan health centers
- To assess knowledge and practice of animal owners about zoonotic brucellosis

2. LITERATURE REVIEW

2.1. Etiology of Brucellosis

The cause of Brucellosis, *brucella* is gram-negative coccobacillus organisms belonging to the Brucelaceae family and genus *Brucella*. Brucella are gram negative, non-sporing, small, round rod-shaped organisms (Pradeepkiran *et al*., 2021). The growth rate of these organisms is relatively slow on ordinary nutrient media, whereas it is improved by serum or blood additions. Species of brucella reside within the reticulo-endothelial and reproductive systems of humans and animals (Godfroid *et al*., 2011). Cells in this species may vary in width from 0.5 to 1.5 μm; they are short and slender, have a straight axis, and have rounded ends and sides. They occur in a single form and are commonly found in pairs (Chachra *et al*., 2012).

The *Brucella* genus has a genetically closely related group of bacteria that are classified into six species based on host preference and biochemical characteristics. *B. abortus, B. melitensis, B. ovis, B. canis, B. suis,* and *B. neotoma* are among the species categorized in this category (Godfroid *et al*., 2011). *B.pinnipediae* and *B. cetacea* which affect marine mammals are recently added (Foster *et al*., 2007). Later classifications were developed based on various characteristics, including different growth abilities on media containing aniline dyes, differences in their agglutination response to monospecific sera, and the capacity to produce hydrogen sulfide (Cloeckaert and Publique, 2018).

2.2. History and Zoonotic Importance of Brucellosis

David Bruce isolated the infectious agent of brucellosis in 1887 from the spleens of soldiers who died of Mediterranean fever on the Isle of Malta (Madkour, 2001). Bruce named the agent “Micrococcus melitensis”. In domestic animals, brucellosis has been referred to as enzootic abortion, contagious abortion, slinking of calves, Bang's disease, infectious abortion or ram epididymitis. In addition to the term brucellosis, in human also reported a variety of other disorders including undulant fever, Cyprus fever, Mediterranean fever, gastric fever, Gibraltar-Rock fever, Malta fever, and Neapolitan fever (Godfroid *et al*., 2005; Moreno, 2014).

Brucellosis is a usually dangerous zoonosis, and infections in humans are caused by direct or indirect contact with animals or animal products, with a few exceptions. The major sources of infection for the human are a dairy product prepared from infected animals which poses great public health consequences. Infected sheep and goat milk may have a huge number of live organisms, which get concentrated in products like soft cheese. which has been identified as a major vehicle of the infection(Yumuk and O'Callaghan, 2012).

2.3. Clinical Signs of Brucellosis

2.3.1. Clinical signs in livestock

Animals who are sexually mature are mainly affected by Brucellosis, which is primarily found in reproductive tissue. Young animals and non-pregnant females are also affected by the illness but do not exhibit clinical symptoms (Megid *et al*., 2010). In livestock, brucellosis affects the reproductive tract as its primary clinical manifestation. Small ruminants and cattle are more likely to develop clinical disease related to reproduction. Late-term abortion or the birth of weak, non-viable calves are both characteristics of the disease in naïve animals but not in endemic herds. When *B. melitensis* is the causative agent of the disease, there is a possibility that cattle do not abort, but their milk will contain bacteria. Human illness may be the only sign of a cattle disease in such a scenario (Radostits *et al*., 2007).

The genital tract and testicles are the primary sites where *brucella* infects bulls. A testicular enlargement and infertility are possible symptoms of the disease if the disease is clearly evident during clinical testing. A testicle may atrophy due to adhesions and fibrosis on rare occasions. Infected bulls commonly develop seminal vesiculitis and ampullitis. Hygromas and arthritis are being observed in cattle rarely (Megid *et al*., 2010).

Both *B. ovis* and *B. melitensis* are found in small ruminants. In *B. ovis* infections, which are primarily infections of sheep, the rams are more affected than the ewes. Rams develop orchitis and epididymitis due to infection and associated inflammation of the epididymis and testes. In ewes, inflammations of placenta can occur that results in the birth of weak lambs, stillbirths, or abortions (Radostits *et al*., 2007). As for infection with *B. melitensis*, the impact of the disease is greater amongst the ewes than the rams and in endemic flocks

retained fetal membrane is commonly found in female animals. Similar to sheep, infected cattle can develop arthritis and hygromas as well Bull develop orchitis, epididymitis, and seminal vesiculitis (Megid *et al*., 2010).

2.3.2. Clinical signs in humans

B. abortus, B. melitensis, B. suis, B. canis, and marine mammal *Brucella* species are human pathogens. In humans, brucellosis can be a serious or occasionally chronic disease that may affect a different organ system. Most cases are caused by occupational exposure to infected animals or the ingestion of unpasteurized dairy products (Shi *et al*., 2018). Humans are an incidental host and the pathogenesis from early infection to phagocytic cell uptake is identical to animal hosts. The lysis of the phagocytic cells that release the *Brucella* organisms also results in the release of cellular debris and pyrogenic endotoxins that cause an episode of fever (Havas, 2011).

In human the infection show febrile illness or undulant fever at the beginning of the disease (Pappas *et al*., 2005). However, the clinical signs in human is not pathognomonic for the disease and include chills, weakness, anorexia, constipation, nervousness, general aches, sweating, headaches, weight loss, and mental depression (Shi *et al*., 2018). Unlike in animals, abortion is not common in human and which may be due to difference in physiology status (Radostits *et al*., 2007).

2.4. Pathogenesis

A host's innate resistance as well as its age, sex, and reproductive status may impact the establishment of the infection, not only the amount of the infective dosage but also the virulence of the bacteria (Radostits *et al*., 2007). *Brucella* organisms can enter the body through ingestion, inhalation, abraded skin penetration, or pharyngeal and alimentary tract mucous membranes. The organisms infect both phagocytes and non-phagocytic cells (Corbel, 2006) , and the latter localize in the rough endoplasmic reticulum (RER). To avoid or suppress macrophage bactericidal responses, an inhibitor produced by *Brucella* organisms prevents phagolysosome fusion, degranulation of myelo-peroxidase and activation of tumor necrosis factor alpha, as well as proliferation of macrophages (Barquero-Calvo *et al*., 2007;Seleem *et al*., 2008).

After invading the host, the organisms are transported to regional lymph nodes by neutrophils and macrophages, where they multiply, causing lymphadenitis. When an organism becomes replicated, it develops bacteremia, which can last for months, reoccurring on a regular basis (Neta *et al*., 2010). Brucella organisms are transported intracellularly or free in the plasma during bacteremia and locate in diverse organs including the gravid uterus and male accessory sex glands (Poester *et al*., 2013). Both antibody-mediated and cellular-mediated responses are evident after *Brucella* infection. The typical time it takes to discover antibodies varies depending on the sex, age, stage of pregnancy, and virulence of the organism (Radostits *et al*., 2007).

Gram-negative bacteria need lipopolysaccharide to function and maintain structural integrity (Neta *et al*., 2010). *Brucella* smooth phenotype is because to the existence of a whole lipopolysaccharide in the outer cell membrane, which is mainly composed of lipid A, a core oligosaccharide, and an O side-chain polysaccharide. Rough (vaccine) strains, those lacking the O-side chain in lipopolysaccharide, are less virulent due to their inability to overcome the host defense system. The lipopolysaccharide of *Brucella* exhibits properties distinct from other lipopolysaccharides. In contrast to classical entero bacterial lipopolysaccharide, those of *Brucella* are several hundred-times less active and less toxic than *Escherichia coli* lipopolysaccharides (Baldwin and Goenka, 2006; Lapaque *et al*., 2006).

According to research, smooth, non-endotoxic lipopolysaccharides help block the development of innate and specific immunity during the early stages of infection and protect the pathogen from the immune system's microbicidal activity (Poester *et al*., 2013). *Brucella melitensis* lipopolysaccharide does not stimulate production of tumor necrosis factor-a or nitric oxide (Zhao *et al*., 2018). *Brucella* LPS plays a role in protecting against bactericidal cationic peptides (defensin NP-2, lactoferrin, cecropines, lysozyme, bactenecin-derived peptides, and the defensin-like antibiotic polymyxin B, and the crude lysosomal extracts from polymorphonuclear leukocytes) (Lapaque *et al*., 2006).

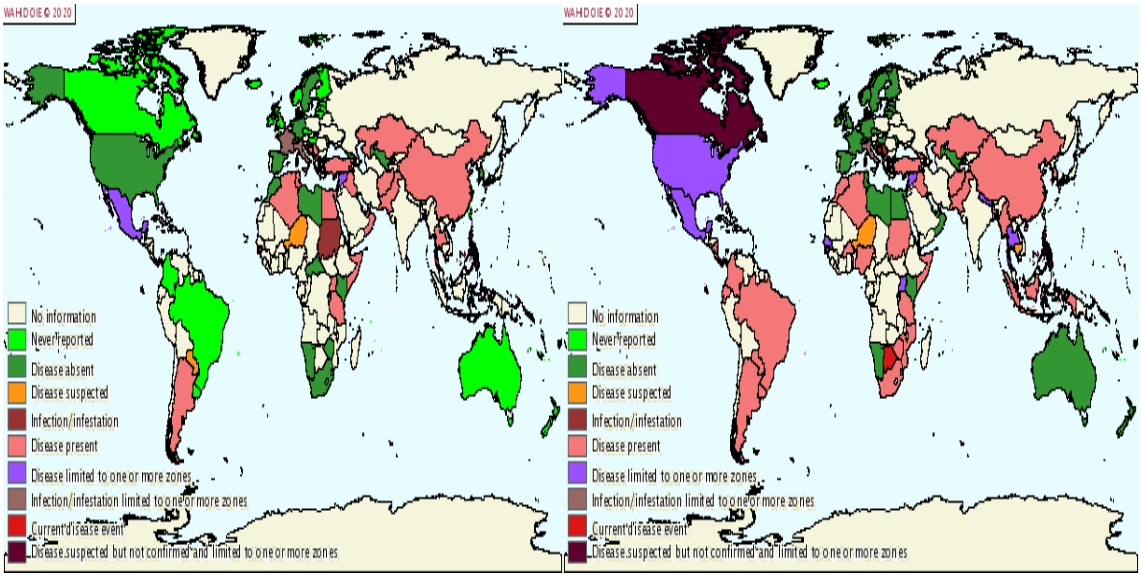
2.5. Epidemiological Distribution

The epidemiology of brucellosis is complicated and continually changing. *Brucella*'s wide host range and resilience to the environment and host immune system help it to persist in population. Infections transmitted to humans come from wild or domesticated animals. Infection can occur through a variety of pathways, including foodborne, occupational, or recreational exposure, travel, and even bioterrorism (Godfroid *et al*., 2011).

2.5.1. World Distribution

Except in countries where bovine brucellosis (*B. abortus*) has been eradicated, the disease is found globally (figure 1). This is defined as a period of at least five years without any reported cases. Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden, and the United Kingdom are among these countries (Seleem *et al*., 2010). Those countries located along the Mediterranean Sea, eastern and northern Africa, Central Asia, Iraq, Afghanistan, Pakistan, Mexico, and South America are still not brucellosis-free (Hull and Schumaker, 2018).

It's impossible to say for sure that *B.melitensis* has ever been eradicated from ruminants in any country, although there are some reports that it has never been detected (Robinson, 2003). However, the incidence of brucellosis in most countries is underreported in spite of it being a nationally notifiable disease. A number of countries are also concerned about brucellosis reemerging (Li *et al*., 2013). Even though brucellosis has been eradicated or is on the verge of being eradicated in many developed countries, it remains a serious human and animal health problem in many parts of the globe, mostly where animal is a main source of food and livelihood (Godfroid *et al*., 2011).



(a) (b)

Figure 1: Worldwide distribution of brucellosis: *B. melitensis* (a) and *B. abortus* (b) report: Source: OIE (2020)

2.5.2. Distribution in Africa.

Africans have had brucellosis in animals and humans for a very long time, as evidenced by a study by Chukwu, (1985). It is a disease of chronic evolution, the importance of which is difficult to assess owing to the dominant livestock production systems of unrestricted grazing, transhumance, and nomadism. However, official Veterinary Services are now starting to turn their attention to brucellosis, after controlling most of the major epizootics that formerly decimated livestock (including rinderpest and contagious pleuropneumonia) and in response to the intensification of dairy livestock production in many Sub-Saharan countries (Matope *et al*., 2011).

African countries where brucellosis was first detected included Zimbabwe (1906), Kenya (1914), and the Orange Free State of South Africa in 1915. Yet little is known about the disease's epidemiology and the best way to prevent it, particularly in Sub-Saharan Africa (Ntirandekura *et al*., 2018). Outside of South Africa, brucellosis surveillance and control were rarely undertaken. In dairy production, the disease is a severe impediment to the importation of high-yielding breeds and a considerable impediment to improving milk production through cross-breeding (Ndazigaruye *et al*., 2016).

In some African countries where the seroprevalence study of the brucellosis had been reported to be greater than 10% are Zambia, 20.7%, Sudan, 24.7%, and Nigeria, 20% (Angara *et al*., 2004; Mai *et al*., 2012; Muma *et al*., 2013). On the other hand, other researchers reported a prevalence rate of 5.5%, 5.4%, 1%, and 2.93% from Zimbabwe, Egypt, Kenya, and Ghana respectively (Kang'ethe *et al*., 2007; Matope *et al*., 2011; Folitse *et al*., 2014; Tesfaye *et al*., 2020) (Table 1).

Table 1: Seroprevalence of brucellosis in ruminants and human reported from selected African countries

Location	**Species**	**Sample size**	**Test used**	**Prev. (%)**	**References**
Rwanda	Cattle	1907	RBPT, iELISA	7.4	(Ntivuguruzwa *et al*., 2020)
	Human	198	RBPT	25	(Gafirita *et al*., 2017)
Cameroon	Cattle	1031	RBPT, iELISA	5.4	(Mouiche *et al*., 2018)
Zambia	Cattle	1712	RBPT, cELISA	7.5	(Mfune *et al*., 2021)
Nigeria	Cattle, goats, sheep	473	RBPT, iELISA	16.3	(Ukwueze *et al*., 2020)
Zimbabwe	Cattle	1291	RBPT, cELISA	5.5	(Matope *et al*., 2011)
Nigeria	goats, sheep	1347	RBPT, SAT	16.1,14.5	(Bertu *et al*., 2010)
Ghana	Cattle	444	RBPT	2.9	(Folitse *et al*., 2014)
Eritrea	sheep	104	RBPT, CFT	1.4	(Omer *et al*., 2000)
Uganda	human	235	SAT, RBPT	17	(Gafirita *et al*., 2017)
Kenya	Human,	1,022	IgG-ELISA	35.8	(Kairu-Wanyoike *et al*., 2019)
	Cattle,	441	c-ELISA	6.3	
	Goats,	961	c-ELISA	3.3	
	Sheep	623	c-ELISA	1.4	

2.5.3. Status of Brucellosis in Ethiopia.

Brucella infection spread is greatly influence by both environmental conditions, as well as, husbandry systems. It has been reported that human and animal prevalence has occurred in different areas of Ethiopia as reported by different authors, which mostly limited to serological studies. Even though several serological surveys have demonstrated the prevalence of brucellosis in Ethiopia, the majority of animal brucellosis research have been conducted in central and northern Ethiopia, and do not provide a comprehensive epidemiological picture of the disease in the country's various agro-ecological zones and livestock production systems (Megersa *et al*., 2011b).

The shreds of evidence of brucellosis in Ethiopian livestock have been serologically demonstrated by different authors (Table 2). According to most studies, seroprevalence was low (< 5%) in ruminants and camels under crop-livestock mixed farming and agro-pastoral systems (Lakew *et al*., 2019; Bifo *et al*., 2020; Bayisa *et al*., 2020; Tesfaye *et al*., 2020) and currently studies on the seroprevalence of bovine brucellosis have been carried out in many parts of Ethiopia by different researchers.

Table 2: Seroprevalence of brucellosis in ruminants and human reported from various parts of Ethiopia

Location	**Species**	**Sample size**	**Test used**	**Prev. (%)**	**reference**
western Ethiopia	Cattle	1,152	RBPT, CFT	1	(Adugna *et al*., 2013)
Sendafa	Cattle	503	RBPT, CFT	0.4	(Bifo *et al*., 2020)
Alage	Cattle	804	RBPT, c-ELISA	2.4	(Asgedom *et al*., 2016)
East and West Shewa Zones	Sheep	1119	mRBPT, CFT	3.5	(Gebremedhin, 2015)
Borana pastoral	Sheep goats	506	RBPT, CFT	0.0	(Tesfaye *et al*., 2020)

Arsi Zone	Cattle	756	RBPT, CFT	2.6	(Tsegaye *et al*., 2016)
Borana pastoral area	Cattle, Sheep and goats	750, 882	RBPT, c-ELISA	2.4, 3.2	(Edao *et al*., 2020)
	Human	341		2.6	
Afar and Somali pastoral areas	Sheep goats	2000	RBPT, I-ELISA	9.7	(Teshale *et al*., 2006)
Fafan Zone of Ethiopian-Somali	Cattle, Sheep, goats Human	268, 108, 172, 211	RBPT, CFT	2.6, 0.9, 2.9, 0.4	(Lakew *et al*., 2019)

2.5.4. Risk factors of Brucella infection

There are so many factors that can affect the pervasiveness of brucellosis in various species of livestock. Brucellosis prevalence varies depending on climatic conditions, geography, species, and age. Asexually mature animals are at risk for brucellosis and *Brucella* is primarily found in the reproductive tract, particularly in pregnant animals; however, there is evidence that the mammary gland may be even more favorable for localization than the reproductive tract (Anonymous, 2007).

In Ethiopia, Researchers found that animal brucellosis is caused by several major risk factors such as increased age and parity, flock size and composition, and history of reproductive health problem (RFM, abortion) across agroecology and production system(Teshale *et al*., 2006; Tsegaye *et al*., 2016). The report by Berhe *et al*. (2007) shown that there was a statistically significant increase in seroprevalence to brucellosis with increasing age and as herd size. Megersa *et al*. (2011a) showed that history of reproductive health problems was more commonly reported in goats 12.4%, cattle 13.8% with seropositivity for anti-*Brucella* antibodies than 1.9%, 2.2% of goats and cattle with out of

the history and flock species composition odds ratio (4.1, 95% CI 1.2-14.2) was found to be risk factors for seropositivity in goats.

People are more likely to contract brucellosis if they handle infected animals, consume contaminated animal products like contaminated milk, meat, travel to endemic areas, and handle *Brucella* cultures in laboratories(Pappas *et al*., 2005).The risk factors can be categorized into those associated with characteristics of the host, the parasite biology, and management (Bamaiyi, 2016).

2.5.5. Sources and transmission of brucellosis

Infected carrier ruminants are the source of brucellosis. Infected cows, does and ewes' reproductive tracts, whether they abort or give birth normally, release vast quantities of *Brucella* in their uterine exudates and placenta. The organism can be present in uterine discharge for at least two months following parturition in infected goats (Radostits *et al*., 2007). Infected animals will excrete the organism in milk in the following lactation, and many will excrete it in all consecutive lactations. Although the organism is excreted from the uterus and milk for a shorter duration in sheep than in goats, the organism can be found in milk during lactation. The duration of excretion in cattle is not known (Ebrahimi *et al*., 2014).

In some cases, brucellosis is transmitted directly from an animal's organ to a human by eating infected, unpasteurized milk products, or by inhaling infected aerosolized particles. In addition, dairy industry workers, abattoir workers, veterinarians, and microbiology laboratory workers are susceptible to brucellosis as a result of their occupations (Pappas *et al*., 2005). Typically, the disease does not spread from person to person, but in some cases, mothers have passed the disease on to their infants during pregnancy or breastfeeding. A very rare case of Brucellosis can be spread via sexual interaction or by contaminated blood or bone marrow transfusions (Franco *et al*., 2007).

Animal brucellosis can be transmitted by both vertical and horizontal transmission. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation, and udder contamination during milking. Congenital infection

during parturition is frequently cleared and only a few animals remained infected as adults (Radostits *et al*., 2007).

2.6. Laboratory Diagnostic methods

Animal disease diagnosis and control must be handled on a herd basis. In some infected animals, the incubation period may be very long, and individuals may not develop a response to infection for a long time afterwards. It is a good indication that infection occurs in the herd if there are one or more infected animals. Additionally, additional animals that are serologically negative may also be incubating the disease and posing a risk. Tests used to diagnose an infection may be divided into two categories: those that prove the organism's presence and those that determine whether it is responding to an antigen (OIE, 2013). The choice of a particular testing strategy depends on the prevailing epidemiological situation of brucellosis in susceptible animals (livestock and wildlife) within a country or region (Godfroid *et al*., 2011).

2.6.1. Bacteriological methods

A direct bacteriological test is the only definitive way to diagnose brucellosis. This test is performed by cultivating *Brucella* from a patient's body fluids (Smirnova *et al*., 2013). However, due to the organism's zoonotic nature, the culture can be risky, necessitating properly equipped laboratories and training. The laboratories must have a bio-safety level three. Further, the time needed to declare a culture negative and the number of false negatives associated with cultures limit the use of this technique. In culture conditions, *Brucella* species grow slowly; they can take up to 45 days to develop (Corbel, 2006).

Brucellosis can be definitively diagnosed through the isolation and identification of Brucella, which may also be useful to track the progress of vaccination programs and for epidemiological purposes. During the collection, transport, and processing of infected materials, adequate precautions must be taken to prevent serious health problems (OIE, 2013). It is more appropriate to culture milk, vaginal discharge, and aborted materials, including the aborted fetus, than blood. In addition, there are several ways to culture

Brucella species, including tissue samples, ascites, pleural fluid, purulent discharge, cerebrospinal fluid, bone marrow, and joint fluid (Corbel, 2006; Franco *et al*., 2007).

2.6.2. Molecular methods

Molecular techniques are important tools for diagnosis and epidemiologic studies, providing relevant information for the Bio-typing of *Brucella* species, which allows the differentiation of vaccine from virulent strains. For Brucella identification and type, molecular biological techniques are often used that are based on polymerase chain reaction amplifications to alleviate the difficulties of bacteriological testing (Nielsen and Yu, 2010; Smirnova *et al*., 2013). As PCR-based identification was initially developed to identify bacterial isolates, this same method can now be used to detect *Brucella* species in human and animal clinical samples without the need to first isolate the organism (Smirnova *et al*., 2013).

Polymerase Chain Reaction: The polymerase chain reaction (PCR) is a widely used diagnostic method for detecting, identifying, and distinguishing many species and strains of *Brucella* (Yu and Nielsen, 2010). Rapid and accurate brucellosis diagnosis can be achieved using PCR without the limitations of conventional methods (Golshani and Buozari, 2017). Brucella Cell Surface 31 kDa protein (bcsp31) and 16S rRNA genes are highly conserved in the Brucella genus, and this principle is used to amplify specific genomic sequences using primers (Bricker *et al*., 2003; Smirnova *et al*., 2013; Yu and Nielsen, 2010). PCR has got many advantages over conventional techniques used for the identification and differentiation of the organism. PCR is performed in less time and results being obtained within a few hours. Furthermore, it does not require the handling of infectious samples, not expensive, and can be automated (Yu and Nielsen, 2010).

Real-time polymerase chain reaction: As compared to traditional PCR methods, real-time PCR provides advanced information regarding the amount of DNA in an individual blood sample, and the ability to automate it. Real-time PCR seems to be highly reproducible, rapid, sensitive, and specific. Additionally, this assay is easily standardized and minimizes the risk of infection in laboratory workers. brucella can be detected using PCR when blood, urine, and paraffin-embedded tissue samples are analyzed (Kattar *et al*., 2007). *Brucella* DNA is detectable in various clinical specimens, including serum, whole

blood and urine samples, different tissues, cerebrospinal fluid, synovial or pleural fluid, and pus. Real-time PCR using the *IS711*, a target sequence that is found in multiple copies within *Brucella* chromosomes, based insertion element assay is the most sensitive, specific, efficient, and reproducible method to detect *Brucella* species (Yu and Nielsen, 2010).

Multiplex polymerase chain reaction: A number of multiplex PCRs exist to diagnose Brucella species or genera and their sub-genus or sub-varieties by combining different primers. Multiplex PCR primers were constructed to detect selected Brucella biovars using the AMOS PCR assay (*abortus-melitensis-ovis-suis*), which uses five oligonucleotide primers. *Brucella* insertion sequence IS711 found in the *Brucella* chromosome is used in this AMOS PCR as it is subject to polymorphism arisen from its status as a species-specific marker (Bricker *et al*., 2003; Kumar *et al*., 2019). However, it has limitations, such as problem identifying some species, such as *B. neotomae* and *B. canis*, and some biovars within a specific species generated negative findings (Scholz and Vergnaud, 2013).

2.6.3. Classical serological methods

The indirect methods of brucellosis diagnostics are based on the detection of the immune response to a bacterial infection. Most of these methods have been initially developed for testing of cattle and then were used to test the domestic goats and sheep (except for the analysis of milk), and later were adapted for the monitoring of certain species of wild animals (Smirnova *et al*., 2013).

In spite of the wide array of serological techniques available today, these tests often fall under the classification of screening, monitoring or epidemiological surveillance, along with complementary or confirmatory methods such as complement fixation, ELISA, and fluorescence polarization. The selection of a given test should take into account the species affected as well as local regulations. In all epidemiological situations, no single serological test is suitable. All serological tests have limitations, particularly when it comes to screening individual animals (Poester *et al*., 2013).

Rose Bengal Plate Test (RBPT): Herds are often tested for brucellosis infection with the rose bengal plate test. The principle of the test depends on an antigen-antibody reaction resulting in agglutination (Kaltungo *et al*., 2014). In a buffered acidic suspension, smooth

Brucella cultures stained with Rose Bengal dye are mixed with an equal volume of serum. The acidic buffer is used to reduce the problems associated with non-specific agglutination (Corbel, 2006). The sensitivity of the test may be influenced by high temperature and consequently, the laboratory is the ideal place to run the test. RBPTs are routinely used as quick screening tests with excellent sensitivity of 99%, but only 68.8% specificity(Poester *et al*., 2013).

Indirect enzyme-linked immunosorbent assay (iELISA): When compared to other serological diagnostic methods, iELISA tests have been recognized and used for their better performance in *Brucella* diagnosis. In many microbiological laboratories, this test is frequently used and accepted. Accordingly, iELISA is likely to be more sensitive than RBPT and CFT for primary binding assays (Tabasi *et al*., 2019). To identify the antigen-antibody combination during the test, most iELISAs use pure *Brucella* antigen but a different anti-bovine Ig conjugate. Despite its high sensitivity, the iELISA test is liable to non-specific results, particularly when *Yersinia enterocolitica* strain (Yo9) infection is present. cELISAs were developed in part to combat cross-reactivity (Godfroid *et al*., 2010).

Competitive enzyme-linked immunosorbent assay (c-ELISA): Anti-*Brucella* antibodies are detected in serum samples from cattle, sheep, goats, and pigs using smooth *Brucella* lipopolysaccharide as an antigen. This test can distinguish between vaccine antibody responses and genuine infections, and its sensitivity ranges from 92 to 100%, while its specificity is between 90 and 99% (Perrett *et al*., 2010). It's also appropriate for both screening and confirmation testing (Poester *et al*., 2013). In all of the tests indicated above, smooth LPS antibodies are employed. They all have one major flaw in common: *Brucella* O-polysaccharides are comparable to those found in other bacteria such as *Yersinia enterocolitica.* As a result, false-positive results occur, lowering the test's specificity (Nielsen and Yu, 2010). The competitive ELISA (cELISA), which uses specific epitopes of *Brucella* O-polysaccharides as antigens, partially solves this problem, although its sensitivity is much lower than that of the iELISA (Nielsen and Yu, 2010).

Complement fixation test (CFT): the complement fixation test is broadly used for the diagnosis of brucellosis in small ruminants and cattle and is of specific value as the means of differentiating between sero-positivity resulting from natural infection and those

produced by vaccination with living vaccine. Cold fixation at 0-4^{o}c for 14 to 18 hours and warm fixation at 37^{o}c for short period of time were the most commonly used techniques for complement fixation (OIE, 2013).

The complement fixation test detects ant-*brucella* antibodies that are able to activate the complement. Even though, it has a good sensitivity and specificity, it is complex method to perform which requires well trained staff and good laboratory facilities to titrate correctly and maintain the reagents (Poester *et al*., 2013). It is very important to titrate each serum sample for the reason that low dilutions of some sera from infected animals do not fix complement which is due to the presence of high levels of non- complement fixing antibody isotypes competing for binding to the antigen. Since these are diluted out at higher dilutions, the complement is fixed (Nielsen and Yu, 2010).

2.7. Treatment, Prevention, and Control of Brucellosis

2.7.1. Treatment

Treatment of brucellosis in animal is rarely recommended or effective when it is undertaken. Among domestic food animals' treatment is not an option given disease eradication goals; thus, infected animals are slaughtered. Brucellosis is usually not treated in animals and the few cases where it has been treated have ended partially successful (Radostits *et al*., 2007).

A significant percentage of Brucella infections result in relapses and treatment failure, due to its in-cell location, its resistance to environmental factors and the requirement for appropriate drug combinations. Due to the high relapse rates associated with monotherapy with single antibiotics, a combination regimen with two antibiotics is the optimal treatment method for brucellosis (Godfroid *et al*., 2005; Seleem *et al*., 2010). Doxycycline plus streptomycin (DS) is currently the best medicine for treating acute and localized forms of brucellosis in terms of side effects and relapse-free outcomes. Neither streptomycin nor doxycycline alone can prevent the multiplication of intracellular *Brucella* (Glynn and Lynn, 2008; Seleem *et al*., 2010).

2.7.2. Prevention and control

The infection brucellosis has been mostly controlled and wiped out in some countries worldwide (Godfroid *et al*., 2010). Due to several factors, including decreasing government budgets and a reduction in disease control operational costs, the provision of animal health services by the public sector across sub-Saharan Africa has decreased greatly over the last 20 years. Sub-Saharan countries don't have sufficient resources, limited information exchange, and no control measures to facilitate coordinated surveillance and monitoring (Franc *et al*., 2018).

Immunization: The most effective method of preventing and controlling livestock brucellosis is vaccination A variety of live and killed vaccines, including *B. melitensis* Rev-1, *B. abortus* strain RB51 *B. suis* S-2, rough *B. melitensis* strain M111 and *B. abortus* 45/20, *B. melitensis* H.38 are used in different countries (Golshani and Buozari, 2017).

The prevention of brucellosis is mainly by control of infection in domestic livestock by mass vaccination. The use *B. abortus* strain S19 in cattle and *B. melitensis* strain Rev-1 in goat and sheep has drastically reduced its incidence in many endemic areas. Vaccination of livestock is relatively cheap and will increase the value and productivity of the animals. It is not only important to improve the health of the animals but also to reduce the risk of severe illness and disability for human and their family members and also reduce the transmission to the human population (Radostits *et al*., 2007).

Application of veterinary extension: The development of national veterinary extension services in the country is crucial to endorse control strategies. The practice of hiding and disposing of animals with an abortion history is prevalent among dairy producers, which could result in disease transmission between farms and areas. Controlling and preventing the disease becomes impossible as a result (Yohannes *et al*., 2012).

By controlling the infection in animals, brucellosis can usually be prevented in humans (Godfroid *et al*., 2011). A vital safety measure in areas of endemicity is the pasteurization of dairy products. Raw or undercooked animal products, as well as unpasteurized dairy products, should not be consumed. Occupational exposure can be prevented by using proper hygiene and protective gear and clothing (Yohannes *et al*., 2012). When assisting

at a birth, performing a necropsy, or butchering an animal for consumption, precautions should be taken to prevent contamination of the skin, inhalation of organisms, and accidental ingestion. Aborted fetuses or their membranes and fluid should be handled carefully. Risky agricultural practices such as crushing the umbilical cord of newborn livestock with the teeth or skinning aborted fetuses should be avoided (OIE, 2013).

3. MATRIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Bako Tibe, Ilu Galan, and Nono districts of West Showa Zone, Oromia regional state, Ethiopia from January 2021 to May 2021(Figure 2). The dominant production system in the districts is mixed crop-livestock production, with extensive management systems. Livestock species mostly cattle, sheep and goats are widely reared in the area although, equines, chicken, and bees are common in the area.

Bako Tibe district is located 251 km to the west of Addis Ababa. The district is positioned at geographical coordinates 9° 8' 0" N and 37°3' 0" E. The total area of the district is about 64,469 hectares of land with an animal population of 138,608 local and 480 exotic cattle, 12,627 sheep, 14,354 goats, 3721 horses, 8,415 donkeys, 8,499 mules, and 97,709 poultry. The area is characterized by having an altitude ranging from 1,300-2,998 meters above sea level, average rainfall of 886.5mm, relative humidity of 57.83%, and an average annual temperature of 21.2°C. The agro-ecology of the area is 52% lowland, 37% midland, and 11% high land (BTLFRDO, 2016).

Ilu Galan district is found in the western part of Ethiopia, in the West Shoa Zone of Oromia Regional State. The district is about 200 km far away to the West of Addis Ababa. It is located at geographical coordinates 8°59'51"N and 37°19'49"E. The mean annual rainfall of the area varies between 1,600-2,290 mm and the annual average temperature is 20.3 °C. The livestock populations of the district based on species were 147,874 cattle, 8,644 sheep, 8,930 goats, 41, 485 poultry, 711 horses, 984 mules, and 5,393 donkeys (IGLFRDO, 2016).

Nono district is located about 210 km southwest of Addis Ababa, in the West Shoa Zone of Oromia National Regional State, Ethiopia. Nono covers an area of over 815.53 km² with an altitude ranging from 1400 to 2250 meters above sea level. It is located at geographical coordinates 8° 32' 0" N, and 37° 26' 0" E and the district has two agro-climatic zones with 70.59% lowland and 29.41% midland. The rainfall ranges of 900-1500mm and the mean annual temperature are 18°C (NLFRDO, 2016).

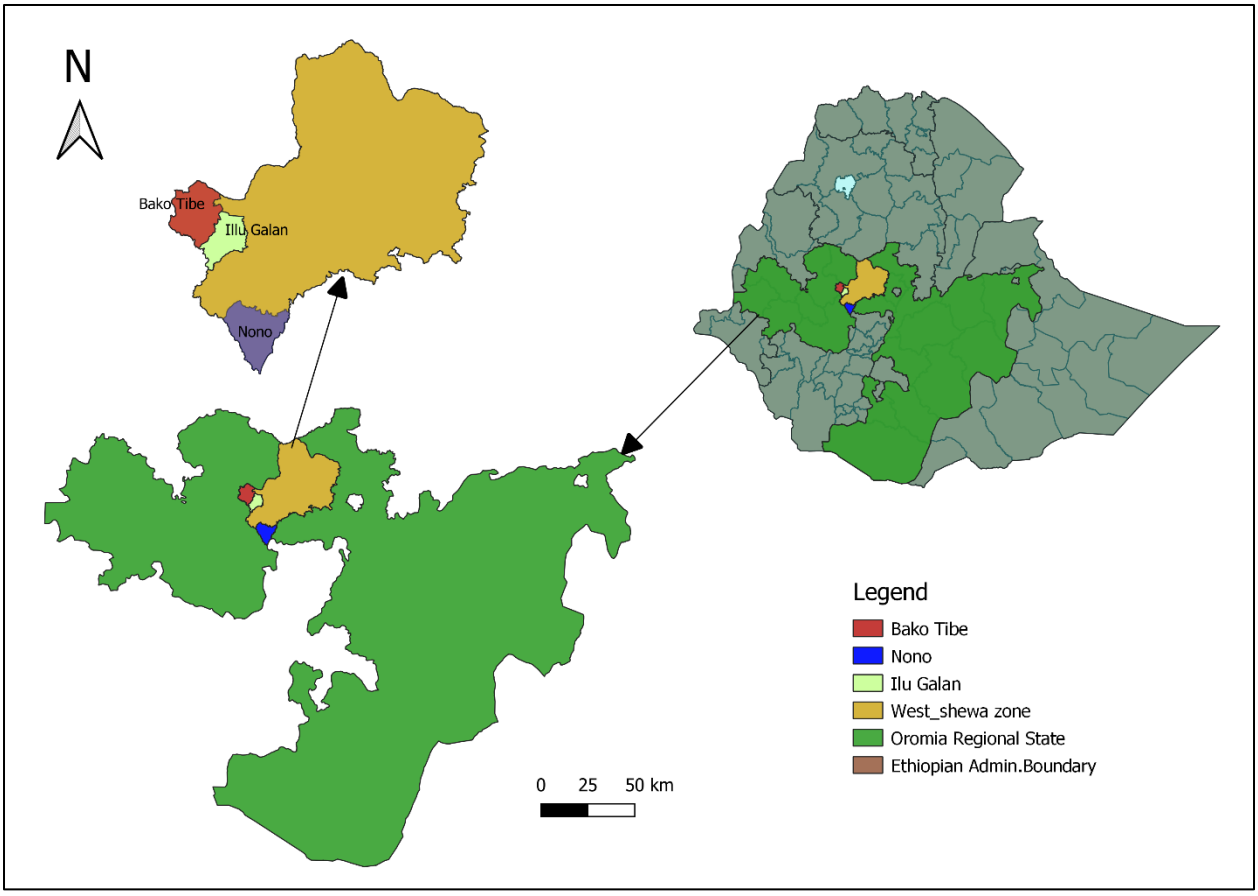


Figure 2: Map of Ethiopia depicting the locations of the study districts

3.2. Study Population

The study populations were local breeds of sheep, goats and cattle managed under a mixed crop-livestock production system in West Shoa, Oromia. Animals of both sexes and all age groups except those younger than six months were sampled. For human participants, all age groups above the age of 15 years old and both sexes were included.

3.3. Study Design

A cross-sectional study was conducted from January 2021 to May 2021 to estimate the seroprevalence, identify the potential risk factors associated with the seroprevalence of brucellosis in animals and assess awareness of participants recruited towards brucellosis

using questionnaire survey. Serum samples from patients admitted to Bako Tibe and Ejaji health centers with malaria negative, non-specific febrile conditions were also used to estimate brucellosis seroprevalence in humans.

3.4. Sampling Procedure and Sample Size Estimation

The purposive sampling technique was applied to select three study districts from the West Shoa Zone based on their agro-ecology. Secondly, kebeles were selected from the selected districts based on abundances of the ruminants and their accessibility. Thirdly, households who rear sufficient number of ruminants and individual animal with in selected households were selected by simple random sampling techniques. Furthermore, human samples were obtained by sampling individuals admitted to the health centers with brucellosis-like symptoms during the study periods after verbal consent was obtained.

The sample size for this study was estimated based on the formula described by Thrusfield, (2018) using a 5% desired precision and confidence interval of 95% by considering an expected prevalence of 3.6% (Bayisa *et al*., 2020) and 2.09 % (Gebremedhin, 2015) in cattle and small ruminants, respectively. Using the same formula, sample size for human was estimated with expected prevalence of 2.15 % (Tibesso *et al*., 2014).

$$n = \frac{1.96^2 P_{exp}\,(1-P_{exp})}{d^2},$$

where: n = sample size required;

P_{exp} = expected prevalence;

d = desired absolute precision.

Hence, using the above formula, the calculated sample size for the current study was 53, 20, 20 heads of cattle, sheep and goats respectively, and 32 humans. In total, however, 295 samples were collected from ruminants during the study which consisting of 170 cattle, 61 sheep, and 64 goats and total of 102 human serum samples were obtained and tested for the presence of *Brucella* antibodies.

3.5. Sample collection and laboratory analysis

3.5.1. Blood sample collection

The blood volume of 7 mL -10 mL from cattle; and 4 mL - 5 mL from sheep and goats were collected using plain vacutainer tubes and needles. Before the withdrawal of blood from the jugular vein, the site was disinfected using 70% alcohol. Numbers were assigned to each animal, and the vacutainer tubes were labelled, and the tubes were tilted 45^{o}overnight to allow the blood to clot. Following centrifugation at 1200 rpm, in chamber temperature, the serum was removed from the clotted blood and syphoned into sterile cryovial tubes in the next morning and were labeled individually. Until tested by rose Bengal plate test and indirect enzyme-linked immunosorbent assay, all collected sera samples were stored at -20 ° C

In the case of sample collection from humans, 4 ml to 5 ml of blood sample was collected aseptically from those patients with the history of fever of unknown origin admitted to both Bako and Ejaji health center. The blood samples were taken from the radial vein by experienced laboratory technicians and informed consent reached before blood sampling. After centrifugation at 1200 rpm for 15 minutes at room temperature, serum was removed from the clotted blood by siphoning into a new sterile screw-capped cryovials tube. The identification was then transferred to the new tube. After sera samples were collected, it was stored at -20 ° C until tested by rose Bengal plate test (RBPT).

3.5.2. Serological tests

To ensure the highest degree of accuracy of test results in epidemiological studies, it is recommended to use two tests serially (Godfroid *et al*., 2010). According to Getachew *et al*., (2016) the sensitivity of RBT, I-ELISA, and CFT was estimated as 89.6% (95% CI: 79.9-95.8), 96.8% (95% CI: 92.3-99.1), and 94% (95% CI: 87.8-97.5) and likewise, specificity was estimated as 84.5% (95% CI: 68-94.98), 96.3% (95% CI: 91.7-98.8), and 88.5% (CI: 81-93.8), for serological diagnosis of brucellosis respectively. Therefore, RBT and I-ELISA were adopted as best combination to screen and confirm the seroprevalence of brucellosis using serum samples.

Primary screening of serum samples for *Brucella* antibody was performed using RBPT as described by (Nielsen and Yu, 2010). Specifically, it used a suspension of Brucella antigen called Rose Bengal antigen (Annex 3). Any visible agglutination was considered positive. For interpretation of the results, both positive and negative control sera were used as recommended by (OIE, 2013). All RBPT positive sera were tested further using commercial brucellosis serum indirect multi-species ELISA Kit (BRUS-MS-5P) Screen Brucellosis Serum Indirect, Multispecies, to detect antibodies directed against *B. abortus*, *B. melitensis,* and *B. suis* at National Animal Health Diagnostic and Investigation Center, Sebata, Ethiopia. The test was performed according to the manufacturer's instructions. It was conducted in 96-well polystyrene plate that were pre-coated with *Brucella* species lipopolysaccharide (LPS) antigen (Annex 4).

3.6. Detection of *Brucella* species from blood samples using PCR

3.6.1. DNA Extraction from blood clot

Genomic DNA extraction from blood clot of an individual blood clot which were previously identified as positive sample with iELISA, Using the QIAMP DNA Mini Kit, DNA was extracted as follows: A 1.5-gram sample of blood clot was taken and mixed with 2 µl of proteinase K and 180 AL buffer for 3 hours at 56^0 C, and then thoroughly mixed with 20 µl of ATL buffer after the lysis process. After transferring the whole volume into the mini spin column, two steps of washing with AW1 and AW2 were carried out. Finally, DNA was eluted with an AE buffer in 100 µl of volume (Annex 5).

3.6.2. Amplification (quantitative RT-PCR)

Each DNA extract was tested using primers targeting the *Brucella*-specific insertion sequence IS711 to detect *Brucella* at the genus level. PCR primers for *Brucella, IS711* gene, were used in the application biosystem PCR system 7500 to amplify extracted DNA, Forward, (5-GCT-TGA-AGC-TTG-CGGACA-G-3) reverse, (5'- GGC-CTA-CCGCTG-GGA-AT 3'), and FAM-AAGCCA-ACA-CCC-GGC-CAT-TAT-GGT-BHQ-1 probe with the internal positive control (IPC). The master mix components were used for *IS711* which consist of forward primer (20µM), reverse primer (20µM), probe (20µM), Taq Man

Universal PCR Master Mix (2x), 10x Exo IPC Mix, 50x Exo IPC DNA and Water to amplify for screening *Brucella* organisms at the genus level (Annex 6).

3.7. Questionnaire survey

Participants were verbally informed of the objectives of the survey before the interview started and given their consent. To gather information from selected volunteer individuals and animal owners who have direct contact with animal and animal products in the study area, the structured questionnaire (Annex 2) was prepared and administered. Data related to demographical characteristics, knowledge level and risky practices were collected.

3.8. Data analysis

Data collected from the field and the serological test was coded and stored in MS excel spreadsheet and transferred to R. software version 3.6.2 for statistical analysis. In order to determine the overall prevalence, the number of RBPT and I-ELISA positive animals were divided by the total number of animals tested. Firth's Bias-Reduced Logistic Regression analysis was used to identify the association of seropositivity with the potential risk factors. The association between risk factors and seropositivity to *Brucella* species was considered significant at $P < 0.05$. Odds ratio (OR) was used to measure the degree of association between risk factors and seroprevalence of brucellosis. Analysis associations among reproductive parameters and seroprevalence of brucellosis were done only for female animals. In addition, a multi-collinearity matrix index and cross-product terms were used to measure the interaction effects before building the final model. In all cases step-wise backward elimination was used to select variables having significant effect on the occurrence of brucellosis. The model validity and predictive abilities were assessed using Hosmer-Leme show goodness of fit test and receiving operating curve (ROC) respectively.

3.9. Ethical considerations and clearance

Ethical clearance certificate was obtained from the animal research ethical review committee of the College of Veterinary Medicine and Agriculture (Date 21/03/021 GC, Ref. No.VM/ERC/05/13/2021) and Oromia Health Bureau (Date 21/7/2013 EC, Ref. No. BEF/HBTFU/146/10103) based on the assessment of the research proposal. The standard

ethical principles and conduct were implemented both in animal and human study participants. Oral informed consents were obtained from human study participants and livestock owners.

4. RESULTS

4.1. Seroprevalence of Brucellosis in Animals

The present study revealed that out of 295 animals tested 3.3 % (n=10) (95 % CI: 1.73-6.34) were found positive by RBPT. The RBPT positive samples were further tested using Indirect ELISA (I-ELISA) for confirmation. Only 4 of the samples gave positive results for brucellosis using I-ELISA yielding an overall prevalence of 1.3 % (95 % CI: 0.43-3.67). Of the sample seropositive on I-ELISA, 1.17% (95 % CI: 0.14 - 4.18), 1.63% (95 % CI: 0.04 - 8.7) and 1.56% (95 % CI: 0.03 - 8.4) were from cattle, sheep and goat respectively.

4.1.1. Potential risk factors of brucellosis

Univariable Firth's Bias-Reduced Logistic Regression analysis was used to evaluate the association between seroprevalence of brucellosis and different risk factors. Out of 295 ruminants screened 4 (3.88 %), 0 (0.00 %), and 0 (0.00 %) were positive from Bako Tibe, Ilu Galan, and Nono districts. Analyses of the data indicate that the origin of the animal and brucellosis were not statistically significant ($P > 0.05$). The seroprevalence of brucellosis was significantly associated with a history of abortion (OR= 26, p=0.003), retained fetal membrane (OR=9, p=0.034), in female animals and animal species composition (OR= 8, p=0.023) in the study area. The seroprevalence was higher (18.18%) in female animals with a history of abortion than those without history of abortion (0.68%).

The seroprevalence of 0.72 % and 1.89 % was observed in male and female animals, although the difference was not statistically significant. Similarly higher prevalence (1.98 %) was observed in animals older than 4 years compared those age 1-2 years whereas similar prevalence was observed among the animal species tested. Higher seroprevalence was recorded in animals having multiple parity (3.4%) than nulliparous ones (0.0%) (OR=2.15, 95 % CI: 0.10- 45.96) as depicted in Table 3.

Table 3: Univariable Firth's bias-reduced logistic regression analysis of risk factors associated with animal brucellosis.

Variables	No. of Animals sampled	No. positive		OR (95%CI)	P-Value
		RBPT (%)	I-ELISA (%)		
Sex					
Male	137	6(4.37)	1(0.72)	1.0	
Female	158	4(2.54)	3(1.89)	2.048 (0.29- 14.06)	0.466
Age					
1-2	74	5(6.75)	1(1.35)	1.0	
2-3	120	3(2.5)	1(0.83)	0.61(0.06 -6.02)	0.676
≥4	101	2(1.98)	2(1.98)	1.23(0.15 - 9.53)	0.842
Species					
Cattle	170	3(1.76)	2(1.17)	1.0	
Sheep	61	3(4.91)	1(1.63)	1.67(0.21-12.92)	0.631
Goats	64	4(6.25)	1(1.56)	1.59(0.20 -12.30)	0.662
Herd size					
small	229	7(3.05)	2(0.87)		
medium	39	2(5.12)	1(2.56)	3.54(0.45 - 27.61)	0.227
large	27	1(3.70)	1(3.70)	5.15(0.654 - 0.56)	0.120
Spp. composition					
mixed	34	3(8.82)	2(5.88)	7.98(1.33 - 47.85)	0.023**
separated	261	7(2.68)	2(0.76)		
Abortion history					
Yes	11	3(27.27)	2(18.18)	25.70(3.06 - 215.83)	0.003**
No	147	1(0.68)	1(0.68)	1.0	
Source					
Brought	21	6(28.57)	1(4.76)	5.67(0.79 - 40.48)	0.083
Homebred	274	4(1.45)	3(1.09)	1.0	

…Continued

Variables	No. of Animals sampled	No. positive		OR (95%CI)	P-Value
		RBPT (%)	I-ELISA (%)		
RFM					
Yes	25	3(12)	2(8)	9.39(1.18 -74.56)	0.034**
No	133	1(0.75)	1(0.75)	1.0	
Parity					
No parity	35	0(0.00)	0(0.00)	1.0	
primiparous	38	1(2.63)	0(0.00)	2.76(0.10 -70.13)	0.537
multiparous	88	3(3.40)	3(3.40)	2.15(0.10- 45.96)	0.624
Districts					
Nono	96	0(0.00)	0(0.00)	1.0	
Ilu Galan	96	1(1.04)	0(0.00)	0.99(0.01- 50.91)	1.00
Bako Tibbe	103	9(8.73)	4(3.88)	8.72 (0.46 -164.30)	0.148

** =statistically significant, OR=Odds ratio, CI=Confidence interval, RFM= retained fetal membrane

Based on the results of a multivariable Firth's Bias-Reduced Logistic Regression analysis, it was found that history of abortion (OR=10.72, 95% CI: 1.06-131.26, P = 0.044) was statistically associated with seroprevalence of brucellosis in female animals, while only herd composition (OR=12.37, 95% CI: 0.98-155.67, P = 0.03) was significant predictor of overall brucellosis in the study area.

Table 4: Multivariable Firth's Bias-Reduced Logistic Regression analysis identifying potential risk factors of brucellosis in ruminants (cattle, sheep, and goat).

Variables	**No. of tested animals**	**I-ELISA (%)**	**OR (95%CI)**	**P-Value**
Spp. composition				
mixed	34	2(5.88)	12.37(0.98 - 155.67)	0.03**
separated	261	2(0.76)		
Abortion history				
Yes	11	2(18.18)	10.72(1.06 - 131.26)	0.044**
No	147	1(0.68)		
RFM				
Yes	25	2(8)	4.69(0.40 - 64.51)	0.204
No	133	1(0.75)		
Source				
Brought	21	1(4.76)	12.90(0.66 - 231.41)	0.084
Homebred	274	3(1.09)		

**=statistically significant, OR=Odds ratio, CI=Confidence interval, RFM= retained fetal membrane

4.2. Molecular detection

The detection of Brucella species was performed with real-time PCR with four blood clots from seropositive samples. The result revealed that no *Brucella* DNA could be isolated from the samples by *IS711* primers.

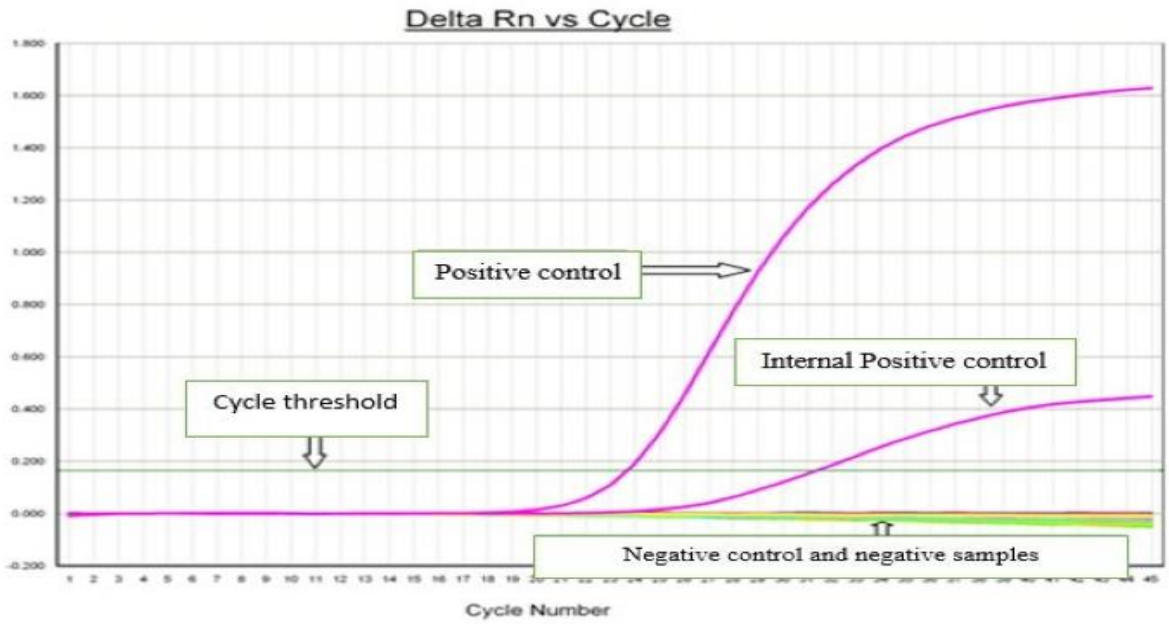


Figure 3: Real time PCR screening test for presence of *Brucella* genus in amplified DNA by *IS711*

4.3. Seroprevalence of Brucellosis in Humans

In this study, Blood samples were taken from 102 consenting individuals brought to Bako and Ejaji town health centers with a history of malaria negative, non-specific febrile state patients by experienced laboratory technicians to assess the status of human brucellosis. The RBPT screening test was performed on all of the sera (n = 102) and all of the samples were found negative for *Brucella* antibodies.

4.4. Results of questionnaire Surveys

4.4.1. Demographic characteristics of the respondents

An overall total of 120 participants (98 men and 28 women) were interviewed to determine their knowledge and practices regarding brucellosis. From the respondents, 26 (21.7 %) had informal education, 37.5 % had primary school, 38.3 % had secondary school and 2.5 % were college graduates. The majority of the respondents (77.5 %) were farmers. Also, 101 (84.2 %) of the participants were married and 19 (15.8 %) were single (figure 4).

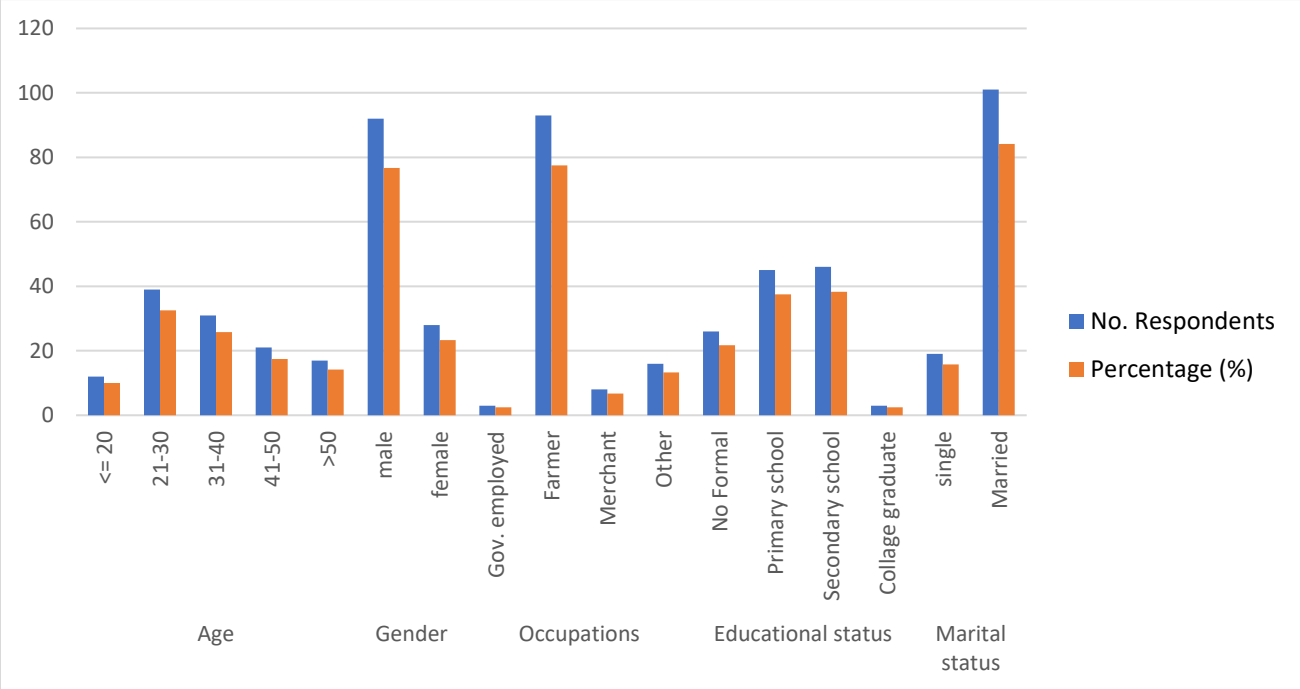


Figure 4: Demographic characteristics of respondents

4.4.2. Respondents' Knowledge, and practices regarding brucellosis

The majority of the respondents to the study, 86 (71.7 %) did not know about brucellosis while 34 (28.3 %) knew something about brucellosis in human and animals. The majority of them 82 (68.3 %) reported that they had a veterinary clinic in their kebeles. They also reported that, they often visit veterinary clinics when pregnant animals are about to give birth 40 (38.3 %). The survey showed that most of the respondents, 82 (68.4 %) had knowledge that the disease is transmitted from animals to humans through handling of

infected animals and their products, and 73 (60.7 %) had not reported consuming raw milk, while 96 (80 %) had the habit of consuming raw meat (Table 5).

Table 5: Knowledge and Practices of respondents about brucellosis.

Variables	**No. Respondents**	**Frequency (%)**
Is there any Vet. Clinic in your Kebele		
No	38	31.7
Yes	82	68.3
If your pregnant animal gets suffer during birth-giving, what do you do?		
Do nothing	14	11.7
Brought to a nearby clinic	46	38.3
Help by myself	60	50
Have you ever heard about brucellosis		
No	86	71.7
Yes	34	28.3
Do you know of any disease transmitted from animal to human through the handling of infected animals and their products?		
No	38	31.6
Yes	82	68.4
Do you consume a raw milk?		
No	73	60.8
Yes	47	39.2
Do you have a habit of consuming a raw meat?		
No	24	20
Yes	96	80
Knowledge on zoonotic diseases transmitted from animals to human		
No	22	18.4
Yes	98	81.6
What do think about the cause abortion in animals		
Infectious	72	60
Non-infectious	48	40

Methods to dispose of aborted material		
Burial	14	11.7
Feeding for dogs	41	34.7
Open damp	65	54.6
Do you clean/disinfect calving areas after parturition properly		
No	62	51.7
Yes	58	48.3

5. DISCUSSION

Since there has been no study done on brucellosis in the current study area, this study provided the first evidence on the occurrence of brucellosis in ruminants and humans in Bako Tibbe district. Although the prevalence observed (1.3 %) is low, its occurrence in animals reared under mixed crop-livestock production system, where small herds are often managed separately, bears significant implication for public health. In the study area, animals especially small ruminants and calves share shelter with their owners. Besides, the low level of knowledge of the interviewed community about brucellosis can accentuate the spread of the bacteria to humans. Therefore, the results of this study could be taken as preliminary report on occurrence of brucellosis in the area by the public health and veterinary authorities.

The overall seroprevalence observed in this study is in agreement with the findings of Degefu *et al*. (2011) from the Agro-pastoral region in Somali regional state, Gumi *et al*. (2013) from Guji and Somali pastoral regions and that of Geresu *et al*. (2016) from Bishoftu and Asella who respectively reported prevalence of 1.3 %, 1.4 %, 1.4 %. In contrary, the prevalence reported in this study is higher than the finding of Bashitu *et al*. (2015) and Edao *et al*. (2018) who reported overall prevalence of 0.2 % in Ambo and Debreberhan and 0.06 % in Addis Ababa. However, other regions of the country showed a relatively high prevalence of brucellosis including the reports of Ibrahim *et al*. (2010) (3.1%); Megersa *et al*. (2011b) (3.5%); Gebremedhin (2015) (3.57%) and Asgedom *et al*. (2016) (2.4%). The differences in seroprevalence observed between this study and the previous ones might be due to differences in herd size, sample size, types of tests used, agroecological and management conditions, and the presence or absence of infectious foci, such as *Brucella*-infected herds, which could spread the disease among contact herds.

The present study revealed that the species composition of ruminants is significantly associated with prevalence of brucellosis. When multi-species of animals are kept together in a herd the odds or risks of acquiring brucellosis become 12.3 times higher compared to the situation in which single species were kept separately. This observation suggests the possibilities of cross-species transmission of *Brucella* organisms. It has been documented

that single *Brucella* species infects multiple hosts and single host species can be infected by multiple *Brucella* species (Megersa *et al*. 2011a; Dadar *et al*. 2019). In consent to our findings multiple livestock species herding, keeping of small ruminants along with cattle, has been reported as a risk factor of *Brucella* infections by Megersa *et al*. (2011b); Kaoud *et al*. (2010) and Tulu and Deresa (2020).

In female animals, history of abortion was statistically significantly associated with the seropositivity to brucellosis conforming to the results of many of previous studies. This suggests that *Brucella* infection can be responsible for abortion in pregnant animals although clinical cases of brucellosis were not encountered during the study period. In agreement with our observation previous authors such as Geresu *et al*. (2016); (Ibrahim *et al*. 2010) and (Tsegaye *et al*. 2016) who reported statistically significant association between brucellosis and abortion. It has documented in the literature that abortion is the typical outcome of brucellosis infections (Anderson, 2007) which is due to *brucella's* preference for the pregnant uterus, in which, erythritol stimulate the growth of these bacteria and elevate in the placenta and fetal fluid during the gestation periods (Radostits *et al*., 2007).

The higher seroprevalence of brucellosis observed among herds in which replacement animals are obtained from market (purchase) compared to those herds which had their own replacement stock suggests the possibility of spread of *Brucella* species in the area. This is discordant with some of previous studies which reported that source of replacement herd had no effect on the occurrence of brucellosis including Jergefa *et al*. (2009); Hordofa (2017); Bifo *et al*. (2020b);Ibrahim *et al*., (2010) and Asmare *et al*. (2013). The fact was due to unrestricted movement of animals through multi-channels of trade within the neighborhood zone around the studies districts. Because herd owners did not maintain a record-keeping system, in most cases the reproductive and health status of the replacement animals was not known.

In the present study, an attempt was made for molecular detection of *Brucella* from serologically positive blood clot samples. However, *Brucella* DNA was not detected using this method from all I-ELISA confirmed blood clots of seropositive ruminants using primers targeting the *Brucella*-specific insertion sequence *IS711*. Failure to detect *Brucella*

from blood clots could be due to the absence of *Brucella* DNA in the sample or the absence of the organisms in the blood clot that was taken and subjected to DNA extraction and amplification. This is in agreement with Corbel, (2006) that absence of the bacteria is not an indicator for the negativity of the animal. Also, being unsuccessful to detect *Brucella* species in all seropositive animals does not mean animals are negative for brucellosis since PCR can result in false negativity (Huber, 2010).

On the other hand, Immunoglobulin G, lactoferrin, and haemoglobin have been recognized as potential PCR inhibitors in blood (Al-Soud and Radstrom, 2001; Sidstedt *et al*., 2020). Recent research by Sidstedt *et al*. (2018) has shown that hemoglobin and immunoglobulin G, were affects amplification efficiency through a direct effect on the DNA polymerase activity and quenches the fluorescence of free dye molecules, and the latter binds to single-stranded genomic DNA, hindering DNA polymerization in the first few PCR cycles. Heparin and ethanol are also known PCR inhibitors that inhibit Taq DNA polymerase and EDTA acts by chelating Mg2+ ions; (Lopata *et al*., 2019).

With regard to human brucellosis, the present study disclosed that none of the human samples tested (n =102) were positive for *Brucella* antibodies, even if they were considered as clinical suspected individuals due to their reported history non-specific febrile condition. This finding is consistent with the earlier reports of Yigeremu, (2006) in Sululta, Ethiopia who tested blood samples collected from occupational exposed groups (abattoir workers and butchers) by RBPT but none of them were positive against *Brucella* antibodies.

Similarly, Kubuafor *et al*., (2000) in Akwapim- South District of Ghana tested blood samples collected from 44 people in high-risk groups and 30 people from control groups but none of them tested positive for the presence of antibodies against *Brucella*. This could be due to the diseases' low prevalence, but it does not rule out the possibility of human infection because only a small proportion of the population was sampled. Another possible reason might be serum from low or non-immune responders may also produce false-negative results, which may underestimate the seroprevalence of brucellosis.

Tibesso *et al*., (2014) on the other hand, reported a seroprevalence of 2.2% after testing 93 sera collected from human patients with non-specific febrile conditions who presented to Adami Tulu health center, as well as those who appeared healthy but were thought to be in

the high-risk group due to a close association with their animals. Similarly, human *Brucella* seropositivity of 0.4% by Lakew *et al*. (2019) and 2.6% by Edao *et al*. (2020) was observed from pastoral settings of Fafan and Borena pastoral communities respectively. Study results probably differ due to factors such as the prevalence of brucellosis in the animal population, the duration of exposure, the size of the samples, the epidemiological characteristics of the population studied, and variations related to the diagnostic test used.

A better understanding of zoonotic infections, including the brucellosis zoonotic infection, among rural communities is likely to have a significant impact on the reduction of several zoonotic infections. A total of 120 livestock owners and attendants were interviewed to assess risk factors that may predispose them to infection and their awareness levels regarding zoonotic brucellosis and risky practices that expose them to the disease using a structured questionnaire.

The present study showed that a sizable proportion of the study respondents 82 (68.4%) perceived certain diseases transmitted from animal to human through the handling of infected animals and their products. But, nearly 71.7 percent of the study respondents had no idea about brucellosis, which means that the majority had never heard of the disease. This finding is similar to those in previous studies conducted in Ghana and Nigeria which found very low awareness of the zoonotic nature of brucellosis (Addo *et al*., 2011; Buhari *et al*., 2015). Other factors, such as an inadequate knowledge of brucellosis and improper handling of aborted animals, can also contribute to further spread of the disease and put public health at risk (Megersa *et al*., 2011b).

The result of the knowledge assessment showed that 60 % of respondents understood as the cause of abortions was non-infectious and close to 90% of them did not bury the aborted material rather, they either left it on the open dump or feed it to dogs. This could have posed a high risk for disease transmission within and between the herd and humans. If abortion in livestock occur due to brucellosis, the fetus and aborted material will be heavily infected by *Brucella* (OIE, 2013). Moreover, 50% of the respondents were assisted during calving and considerable proportions (48.3%) of them do not clean/disinfect the calving areas properly after parturition. This is in line with earlier reports of Lindahl *et al*., (2015); Geresu *et al*., (2016).

6. CONCLUSION AND RECOMMENDATIONS

The current study found antibodies to *Brucella* species in cattle, sheep, and goats living in the same ecological zone, suggests the possibilities of cross-species transmission of *Brucella* organisms. Thus, the presences of brucellosis in apparently healthy animals, indicating that these animals are reproducing normally and serve as permanent carriers of brucellosis which bears significant public health implications. There were statistically significant risk factors related to animal brucellosis in this study, including the history of abortion in female animals and species composition for overall brucellosis in the study. None of the human samples were found positive for *Brucella* antibodies. This study also revealed important information on livestock owners' knowledge and practices regarding brucellosis which resulted in significant zoonotic importance like assisting pregnant animals during calving. Farmers may be at a higher risk of infection due to their lack of understanding and increasing contact with their livestock. The following suggestions are worth highlighting based on the current findings:

- To understand the transmission dynamics of *Brucella* species, more broad epidemiological investigations involving one health approach are needed to isolate and characterize the *brucella* species circulating in animal and human population.
- To decrease the risk of brucellosis transmission, different livestock species and aborted animals should always be housed and managed separately.
- Traditional practices that may expose livestock owners and the general public to *Brucella* infection are needed to be addressed to enhance their awareness level of the communities.

7. REFERENCES

Addo, K., Mensah, G., Nartey, N., Nipah, G., Mensah, D., Aning, G., Smits, H., (2011): Knowledge, Attitudes and Practices (KAP) of herdsmen in Ghana with respect to milk-borne zoonotic diseases and the safe handling of milk. *J. Basic Appl. Sci. Res.* **1**: 1556 -1562.

Adugna, K., Agga, G., Zewde, G. (2013): Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. *Rev Sci Tech.* **32**: 765-773.

Ahmed, W., Majeed, S., Ameer, A., Mahmmod, N., Saeed, N., Hanaa, L. (2016): Sensitivity and specificity of various serological tests for detection of *Brucella* spp. infection in male goats and sheep. *Adv. Microbiol.* **6**: 98-103.

Al-Soud, W., Radstrom, P. (2001): Purification and characterization of PCR-inhibitory components in blood cells. *J. Clin. Microbiol.* **39**: 485-493.

Anderson, M.L. (2007): Infectious causes of bovine abortion during mid-to late-gestation. *Theriogenology.* **68**: 474-486.

Angara, T., Ismail, A., Agab, H., Saeed, N. (2004): Sero-prevalence of bovine brucellosis in Kuku Dairy Scheme, Khartoum North, Sudan. College of Veterinary Medicine and Animal Production, Sudan University of Science and Technology.1-17

Anonymous, (2007): Brucellosis background. American Veterinary Medical Association. www.avma.org/public_health/brucellosis_ bgnd

Asfaw, Y., Molla, B., Zessin, K., Tegegne, A. (1998) A cross-sectional study of bovine brucellosis and test performance in intra-and peri-urban production systems in and around Addis Ababa, Ethiopia. *Bull. anim. Hlth. Prod. Afr*. **46**: 217-224.

Asgedom, H., Damena, D., Duguma, R. (2016): Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district , Ethiopia. *Springerplus* **5**: 1-8.

Asmare, K., Sibhat, B., Molla, W., Ayelet, G., Shiferaw, J., Martin, A.D., Skjerve, E., Godfroid, J. (2013): The status of bovine brucellosis in Ethiopia with special emphasis on exotic and cross bred cattle in dairy and breeding farms. *Acta. Trop*. **126**: 186-192.

Baldwin, C., Goenka, R. (2006): Host immune responses to the intracellular bacteria

Brucella: does the bacteria instruct the host to facilitate chronic infection? *Crit. Rev. Immunol.* **26**: 521-538

Bamaiyi, P.H. (2016): Prevalence and risk factors of brucellosis in man and domestic animals: A review Prevalence and risk factors of brucellosis in man and domestic. *Int. J. One Heal.* **2**: 29-34.

Barquero-Calvo, E., Chaves-Olarte, E., Weiss, D., Guzmán-Verri, C., Chacón-Díaz, C., Rucavado, A., Moriyón, I., Moreno, E. (2007): *Brucella* abortus uses a stealthy strategy to avoid activation of the innate immune system during the onset of infection. *PLoS One*. **2**: 6-31.

Bashitu, L., Afera, B., Tuli, G., Aklilu, F. (2015): Sero-Prevalence Study of Bovine Brucellosis and its Associated Risk Factors in Advances in Dairy Research. *Adv. Dairy Res.* **3**: 1-4.

Bayisa, S., Bulcha, B., Waktole, H., Abunna, F., Tuli, G. (2020): The Sero-Prevalence Study of Bovine Brucellosis and Its Risk Factors. *Inter. Journal. Medic. Vet. Sci* **1**: 32-44.

Bekele, T., Kasali, O. (1990): Brucellosis in sheep and goats in Central Ethiopia. *Bull. Anim. Heal. Prod. Africa.* **38**: 23-25.

Berhe, G., Belihu, K., Asfaw, Y. (2007): Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. *Int. J. Appl. Res. Vet. Med.* **5**: 65-71.

Bertu, W., Ajogi, I., Bale, J., Kwaga, J., Ocholi, R. (2010): Sero epidemiology of brucellosis in small ruminants in Plateau State, Nigeria. *Afri. J. Microbiol. Res.* **4**: 1935-1938.

Bifo, H., Gugsa, G., Kifleyohannes, T., Abebe, E., Ahmed, M. (2020): Sero-prevalence and associated risk factors of bovine brucellosis in Sendafa , Oromia Special. *PLoS One.* **15**: 1-12.

Bricker, B., Ewalt, D., Olsen, S., Jensen, A. (2003): Evaluation of the *Brucella* abortus species specific polymerase chain reaction assay, an improved version of the *Brucella* AMOS polymerase chain reaction assay for cattle. *J. Vet. Diagn. Investig*. **15**: 374-378.

BTLFRDO. (2016): Bako Tibe livestock and fishery resource development office. *The*

annual report. Bako Tibe, Ethiopia.

Buhari, H., Saidu, S., Mohammed, G., Raji, M. (2015): Knowledge, attitude and practices of pastoralists on bovine brucellosis in the north senatorial district of Kaduna state, Nigeria. *J. Anim. Heal. Prod.* **3**: 28-34.

Chachra, D., Kaur, H., Chandra, M., Saxena, H. (2012): Isolation, electron microscopy and physicochemical characterization of a *Brucella*phage against *Brucella* abortus vaccine strain S19. *Intern. J.Microbiol.* **10**: 1-7.

Chukwu, C. (1985): Brucellosis in Africa. I. The prevalence. Bull. Anim. Heal. Prod. Africa Bull. des sante Prod. *Anim. en Afrique*. **33**: 193-198.

Cloeckaert, A., Publique, S. (2018): *Brucella*:Bergey's Manual of Systematics ofArchaea and Bacteria. pp:1-38.

Corbel, M. (2006): Brucellosis in humans and animals. World Health Organization. Produced by the, WHO in collaboration with the, FAO and OIE, Geneva.pp.1-87.

Dadar, M., Alamian, S., Behrozikhah, A., Yazdani, F., Kalantari, A., Etemadi, A., Whatmore, A. (2019): Molecular identification of *Brucella* species and biovars associated with animal and human infection in Iran, in: *Veterinary Research Forum*. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, 10: 315-32.

Degefu, H., Mohamud, M., Hailemelekot, M., Yohannes, M. (2011): Seroprevalence of bovine brucellosis in agro pastoral areas of Jijjiga zone of Somali Regional State, Eastern Ethiopia. *Ethiop. Vet. J.* **15**: 37-47.

Ebrahimi, A., kanluye Milan, J., Mahzoonieh, M., Khaksar, K. (2014): Shedding rates and seroprevalence of *Brucella* melitensis in lactating goats of shahrekord, Iran. *Jundishapur J. Microbiol*. 7:1-4.

Edao, B., Ameni, G., Assefa, Z., Berg, S., Whatmore, M., Wood, J. (2020): Brucellosis in ruminants and pastoralists in Borena, Southern Ethiopia. *PLoS Negl. Trop. Dis.* **14**: 1-17.

Edao, B., Hailegebreal, G., Berg, S., Zewude, A., Zeleke, Y., Sori, T., Almaw, G., Whatmore, A., Ameni, G., Wood, J. (2018): Brucellosis in the Addis Ababa dairy cattle: the myths and the realities. *BMC Vet. Res.* **14**: 1-9.

Folitse, R., Boi-Kikimoto, B., Emikpe, B., Atawalna, J. (2014): The prevalence of Bovine tuberculosis and brucellosis in cattle from selected herds in Dormaa and Kintampo

Districts, Brong Ahafo region, Ghana. *Arch. Clin. Microbiol.* **5**:2-9.

Foster, G., Osterman, B.S., Godfroid, J., Jacques, I., Cloeckert, A. (2007): *Brucella* ceti sp. nov. and *Brucella* pinnipedialis sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. *Int. J. Syst. Evol. Microbiol.* **57**: 2688-2693.

Franc, K., Krecek, R., Häsler, B., Arenas-Gamboa, A. (2018): Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health.* **18**: 1-9.

Franco, M., Mulder, M., Gilman, R., Smits, H. (2007): Human brucellosis. *Lancet Infect. Dis.* **7**: 775-786.

Gafirita, J., Kiiza, G., Murekatete, A., Ndahayo, L., Tuyisenge, J., Mashengesho, V., Ruhirwa, R., Nyandwi, T., Asiimwe-Kateera, B., Ndahindwa, V., Njunwa, K. (2017): Seroprevalence of brucellosis among patients attending a District Hospital in Rwanda. *Am. J. Trop. Med. Hyg.* **97**: 831-835.

Gebremedhin, E.Z. (2015): Seroepidemiology of Ovine Brucellosis in East and West Shewa Zones of Oromia Regional State. *J. Vet. Sci. Technol.* **6**: 1-8.

Genene, R., Desalew, M., Yamuah, L., Hiwot, T., Teshome, G., Asfawesen, G., Abraham, A., Abdoel, T., Smits, H. (2009): Human brucellosis in traditional pastoral communities in Ethiopia. *Int. J. Trop. Med.* **4:** 59-64.

Geresu, M., Ameni, G., Tuli, G., Arenas, A., Kassa, G. (2016): Seropositivity and risk factors for *Brucella* in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia. *African J. Microbiol. Res.* **10**: 203-213.

Getachew, T., Getachew, G., Sintayehu, G., Getenet, M., Fasil, A., 2016. Bayesian estimation of sensitivity and specificity of rose bengal, complement fixation, and indirect ELISA tests for the diagnosis of bovine brucellosis in Ethiopia. *Vet. Med. Int.* **2016**:1-5.

Glynn, M., Lynn, T. (2008): Zoonosis update. *A.V.M.A.* **233**: 900-908.

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

Godfroid, J., Nielsen, K., Saegerman, C. (2010): Diagnosis of brucellosis in livestock and

wildlife. *Croat. Med. J.* **51**: 296–305.

Godfroid, J., Scholz, H., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., Whatmore, A., Cloeckaert, A., Blasco, J., Moriyon, I., Saegerman, C., Muma, J., Dahouk, S. Al, Neubauer, H., Letesson, J. (2011): Brucellosis at the animal / ecosystem / human interface at the beginning of the 21st century. *Prev. Vet. Med.* **102**: 118-131.

Golshani, M., and Buozari, S. (2017): A review of brucellosis in Iran: epidemiology, risk factors, diagnosis, control, and prevention. *Iran. Biomed. J.* **21**: 349-359.

Gumi, B., Firdessa, R., Yamuah, L., Sori, T., Tolosa, T., Aseffa, A., Zinsstag, J., Schelling, E. (2013): Seroprevalence of brucellosis and Q-fever in southeast Ethiopian pastoral livestock. *J. Vet. Sci. Med. Diagn*. **2**:1-11

Havas, K. (2011): Dissertation a systemic review of brucellosis in the kakheti region of the country of Georgia: an evaluation of the disease ecology, risk factors and suggestions for disease control. *PhD thesis*. pp.1-254

Hordofa, K., (2017): Sero- Epidemiological study of bovine brucellosis in selected dairy farms of Bishoftu and Holeta towns, Oromia regional state, central Ethiopia, *MVSc Thesis.* Addis Ababa University.pp.1-129

Huber, B. (2010): Development of diagnostic PCR assays for typing and subtyping of the highly pathogenic bacterial taxa *Brucella*, *Francisella* and *Yersinia pestis*. *Dissertation*, University of Vienna. pp.1-175

Hull, N., Schumaker, B. (2018): Comparisons of brucellosis between human and veterinary medicine. *Infect. Ecol. Epidemiol.* **8:** 1500846.

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

IGLFRDO. (2016): Ilu Galan livestock and fishery resource development office annual report. *The annual report*. Ilu Galan, Ethiopia.

Jergefa, T., Kelay, B., Bekana, M., Teshale, S., Gustafson, H., Kindahl, H. (2009): Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. *Rev. sci. tech. Off. int. Epiz* **28**: 933-943.

Kairu-Wanyoike, S., Nyamwaya, D., Wainaina, M., Lindahl, J., Ontiri, E., Bukachi, S., Njeru, I., Karanja, J., Sang, R., Grace, D. (2019): Positive association between

Brucella spp seroprevalences in livestock and humans from a cross-sectional study in Garissa and Tana River Counties, Kenya. *PLoS Negl. Trop. Dis.* **10**: 1-15.

Kaltungo, B., Saidu, S., Sackey, A., Kazeem, H. (2014): A review on diagnostic techniques for brucellosis. *African J. Biotechnol.* **13**: 1-10.

Kang'ethe, E., Ekuttan, C., Kimani, V., Kiragu, M. (2007): Investigations into the prevalence of bovine brucellosis and the risk factors that predispose humans to infection among urban dairy and non-dairy farming households in Dagoretti Division, Nairobi, Kenya. *East Afr. Med. J.* **84**: 96-100.

Kaoud, H., Zaki, M., El-Dahshan, A., Nasr, S. (2010): Epidemiology of brucellosis among farm animals. *Nat. Sci.* **8**: 190-197.

Kattar, M., Zalloua, P., Araj, G., Samaha-Kfoury, J., Shbaklo, H., Kanj, S., Khalife, S., Deeb, M. (2007): Development and evaluation of real-time polymerase chain reaction assays on whole blood and paraffin-embedded tissues for rapid diagnosis of human brucellosis. *Diagn. Microbiol. Infect. Dis.* **59**: 23-32.

Kebede, T., Ejeta, G., Ameni, G. (2008): Seroprevalence of bovine brucellosis in small holder farms in central Ethiopia (Wuchale-Jida district). *Rev. Med. Vet*. **159**: 3-9.

Kubuafor, D., Awumbila, B., Akanmori, B., (2000): Seroprevalence of brucellosis in cattle and humans in the Akwapim-South district of Ghana: public health implications. *Acta Trop.* **76**: 45-48.

Kumar, V., Bansal, N., Nanda, T., Kumar, A., Kumari, R., Maan, S. (2019): PCR based molecular diagnostic assays for brucellosis: a review. *Int. J. Curr. Microbiol. App. Sci.* **8**: 2666-2681.

Lakew, A., Hiko, A., Abraha, A., Hailu, S. (2019): Sero-prevalence and community awareness on the risks associated with Livestock and Human brucellosis in selected districts of Fafan Zone of Ethiopian-Somali National Regional State. *Vet. Anim. Sci.* **7**: 1-6.

Lapaque, N., Forquet, F., De Chastellier, C., Mishal, Z., Jolly, G., Moreno, E., Moriyon, I., Heuser, J.E., He, H., Gorvel, J. (2006): Characterization of *Brucella* abortus lipopolysaccharide macrodomains as mega rafts. *Cell. Microbiol.* **8**: 197-206.

Li, Y., Li, X., Liang, S., Fang, L., Cao, W., 2013. Epidemiological features and risk factors associated with the spatial and temporal distribution of human brucellosis in China.

BMC infect. dis. **13**: 1-12

Lindahl, E., Sattorov, N., Boqvist, S., Magnusson, U. (2015): A Study of Knowledge , Attitudes and Practices Relating to Brucellosis among Small-Scale Dairy Farmers in an Urban and Peri-Urban Area of Tajikistan. *PLoS ONE*. **10**: 1-10.

Lopata, A., Jójárt, B., Surányi, É., Takács, E., Bezúr, L., Leveles, I., Bendes, Á.Á., Viskolcz, B., Vértessy, B., Tóth, J. (2019): Beyond Chelation: EDTA Tightly Binds Taq DNA Polymerase, MutT and dUTPase and Directly Inhibits dNTPase Activity. *Biomolecules.* **9**: 1-19.

Madkour, M.M. (2001): Historical aspects of brucellosis, in: Madkour's Brucellosis. *Springer*, pp. 15-20.

Mai, H., Irons, P., Kabir, J., Thompson, P. (2012): A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. *BMC Vet. Res*. **8**: 1-14.

Matope, G., Bhebhe, E., Muma, J.B., Lund, A., Skjerve, E. (2011): Risk factors for *Brucella* spp. infection in smallholder household herds. *Epidemiol. Infect.* **139**: 157-164.

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

Megersa, B., Biffa, D., Niguse, F., Rufael, T., Asmare, K., Skjerve, E. (2011b): Cattle brucellosis in traditional livestock husbandry practice in Southern and Eastern Ethiopia, and its zoonotic implication. *Acta. Vet. Scand*. **53**: 1-8.

Megid, J., Mathias, L., Robles, C. (2010): Clinical manifestations of brucellosis in domestic animals and humans. *Open Vet. Sci. J.* **4**: 119-126.

Mfune, R., Godfroid, J., Bernard, M., Muma, J. (2021): Seroprevalence of Bovine Brucellosis in Selected Districts of Zambia Seroprevalence of bovine brucellosis in selected districts of Zambia. *Int. J. Environ. Res. Public Heal.* **18***:* 1-8

Mouiche, M., Bayang, H., Ngwa, V., Assana, E., Feussom, K., Manchang, T., Zoli, P. (2018): Seroprevalence and Associated Risk Factors of Brucellosis among Indigenous Cattle in the Adamawa and North Regions of Cameroon. *Vet. Med. Int*. **18**:1-13.

Muma, J., Syakalima, M., Munyeme, M., Zulu, V., Simuunza, M., Kurata, M. (2013): Bovine tuberculosis and brucellosis in traditionally managed livestock in selected districts of Southern Province of Zambia. *Vet. Med. Int.* **2013**: 1-7.

Ndazigaruye, G., Mushonga, B., Kandiwa, E., Samkange, A., Segwagwe, B.E., Segwagwe, B.E., Province, E. (2016): Prevalence and risk factors for brucellosis seropositivity in cattle in Nyagatare District , Eastern Province , Rwanda. *J.S.Afr.Vet.Assoc*. **89**: 1-8.

Neta, A., Mol, J., Xavier, M., Paixão, T., Lage, A., Santos, R. (2010): Pathogenesis of bovine brucellosis. *Vet. J.* **184**: 146-155.

Nielsen, K., Yu, W. (2010): Serological diagnosis of brucellosis. *Prilozi.* **31**: 65–89.

IGLFRDO. (2016): Ilu Galan livestock and fishery resource development office annual report. *The annual report*. Ilu Galan, Ethiopia.

Ntirandekura, J., Matemba, L., Kimera, S., Muma, B., Karimuribo, E., (2018): Association of Brucellosis with Abortion Prevalence in Humans and Animals in Africa: A Review. *Afr. J. Reprod. Heal*. **22**: 120-136.

Ntivuguruzwa, J., Kolo, F., Gashururu, R., Umurerwa, L., Byaruhanga, C., Heerden, H. Van. (2020): Seroprevalence and Associated Risk Factors of Bovine Brucellosis at the Wildlife-Livestock-Human Interface in Rwanda. *microorganisms.* **8**: 1-15.

OIE, (2013): Manual of diagnostic tests and vaccines for terrestrial animals. collection, submission and storage of diagnostic specimens. *OIE Terr. Man.* P:1-104.

Omer, M., Skjerve, E., Holstad, G., Woldehiwe, T., Macmillan, A. (2000). Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems. *Epidemiol. Infect.* **125**: 447-453.

Pal, M., Gizaw, F., Fekadu, G., Alemayehu, G., Kandi, V. (2017): Public Health and Economic Importance of Bovine Brucellosis: An Overview. *Am. J. Epidemiol. Infect. Dis.* **5**: 27-34.

Pappas, G., Akritidis, N., Bosilkovski, M., Tsianos, E. (2005): medical progress Brucellosis: Review Article. *N. Engl. J. Med*. **352**: 2325-2336.

Perrett, L., McGiven, J., Brew, S., Stack, J. (2010): Evaluation of competitive ELISA for detection of antibodies to *Brucella* infection in domestic animals. *Croat. Med. J.* **51**: 314-319.

Pieracci, E., Hall, A., Gharpure, R., Haile, A., Walelign, E., Deressa, A., Bahiru, G.,

Kibebe, M., Walke, H., Belay, E. (2016) Prioritizing zoonotic diseases in Ethiopia using a one health approach. *ONEHLT*. **2**: 131-135.

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

Poester, P., Nielsen, F., Samartino, K., Luis Ling Yu, W., Yu, L., Wei, (2013): Diagnosis of brucellosis. *Open Vet. Sci. J.* **4**: 1-6.

Pradeepkiran, J., Bhaskar, M., Shrikanya, K., Krishna, P., Reddy, M, Venkatrayulu, C., Sainath, S.B. (2021): Introduction to brucellosis, in: *Brucella* Melitensis. Elsevier, pp. 1-23.

Radostits, O., Gay, C., Hinchcliff, K., Constable, P. (2007): A textbook of the diseases of cattle, horses, sheep, pigs and goats. Veterinary medicine. 10th ed., pp. 2045-2050.

Robinson, A. (2003). Guidelines for coordinated human and animal brucellosis surveillance, FAO animal production and health paper. FAO Rome, Italy.pp.1-45.

Scholz, H.C.and Vergnaud, G. (2013). Molecular characterisation of *Brucella* species. *Rev. Sci. Tech.* **32**: 149-162.

Seleem, M., Boyle, S., Sriranganathan, N. (2010) Brucellosis: a re-emerging zoonosis. *Vet. Microbiol.* **140**: 392-398.

Seleem, M.N., Boyle, S.M., Sriranganathan, N. (2008): *Brucella*: a pathogen without classic virulence genes. *Vet. Microbiol.* **129**: 1-14.

Shi, Y., Gao, H., Pappas, G., Chen, Q., Li, M., Xu, J., Lai, S., Liao, Q., Yang, W., Yi, Z., (2018): Clinical features of 2041 human brucellosis cases in China. *PLoS One*. **13**: e0205500.

Sidstedt, M., Hedman, J., Romsos, E.L., Waitara, L., Wadsö, L., Steffen, C.R., Vallone, P.M., Rådström, P. (2018): Inhibition mechanisms of hemoglobin , immunoglobulin G , and whole blood in digital and real-time PCR. *Anal. Bioanal. Chem.* **410**: 2569-2583.

Sidstedt, M., Rådström, P., Hedman, J. (2020): PCR inhibition in qPCR , dPCR and MPS mechanisms and solutions. *Anal. Bioanal. Chem.* **412**: 2009-2023.

Sintayehu, G., Melesse, B., Abayneh, D., Sintayehu, A., Melaku, S., Alehegne, W., Mesfin, S., De Blas, I., Casal, J., Allepuz, A. (2015): Epidemiological survey of brucellosis in sheep and goats in selected pastoral and agro-pastoral lowlands of Ethiopia. *Rev. Sci.*

Tech. **34**: 881-893.

Smirnova, E., Vasin, A., Sandybaev, N., Klotchenko, S., Plotnikova, M., Chervyakova, O, Sansyzbay, A., Kiselev, O. (2013): Current methods of human and animal brucellosis diagnostics. *Adv. Infect. Dis.* **3**: 1-8.

Tabasi, M., Eybpoosh, S., Bouzari, S. (2019): Development of an indirect ELISA based on whole cell *Brucella* abortus S99 lysates for detection of IgM anti-*Brucella* antibodies in human serum. *Comp. Immunol. Microbiol. Infect. Dis*. **63**: 87–93.

Tesfaye, A., Sahele, M., Sori, T., Guyassa, C., Garoma, A. (2020): Seroprevalence and associated risk factors for chlamydiosis, coxiellosis and brucellosis in sheep and goats in Borana pastoral area, southern Ethiopia. *BMC Vet. Res*. **16**: 1-8.

Teshale, S., Endalew, A., Gelaw, A., Africa, S. (2006): Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia: the impact of husbandry. *Rev. Med. Vet*. **157**: 557-563.

Thrusfield, M. (2018): Veterinary epidemiology, 4th ed. John Wiley & Sons.

Tibesso, G., Ibrahim, N., Tolosa, T., (2014) Sero-Prevalence of Bovine and Human Brucellosis in Adami Tulu, Central Ethiopia. *World Appl. Sci. J.* **31**: 776-780.

Tsegaye, Y., Kyule, M., Lobago, F. (2016): Seroprevalence and risk factors of bovine brucellosis in Arsi Zone, Oromia Regional State, Ethiopia. *Am. Sci. Res. J. Eng. Technol. Sci.* **24**: 16-25.

Tulu, D., Deresa, B. (2020): Epidemiological investigation of brucellosis in breeding female cattle under the traditional production system of Jimma zone in Ethiopia. *Vet. Anim. Sci*. **9**: 100-117.

Ukwueze, K., Ishola, O., Dairo, M, Awosanya, E., Cadmus, S. (2020): slaughtered in Oko-Oba abattoir, Lagos State, southwestern Nigeria. *Pan Afri. Med. J.* **36**: 1-11.

Workalemahu, B., Sewunet, T., Astatkie, A. (2017): Seroepidemiology of Human Brucellosis among Blood Donors in Southern Ethiopia: Calling Attention to a Neglected Zoonotic Disease. *Am. J. Trop. Med. Hyg.* **96**: 88-92.

Yigeremu, M. (2006): Study on bovine brucellosis in cattle slaughtered at Addis Ababa and Sululta abattoirs with focus on occupational hazard. *MSc Thesis*.pp.1-114

Yohannes, M., Tesfaye, M., Hailu, D., Tadele, T., Mezene, W. (2012): Bovine brucellosis: serological survey in Guto-Gida district, East Wollega zone, Ethiopia. *Glob. Vet*. **8**:

139-143.

Yu, W., Nielsen, K. (2010): Review of detection of *Brucella* spp. by polymerase chain reaction. *Croat. Med. J.* **51**: 306-313.

Yumuk, Z., O'Callaghan, D. (2012): Brucellosis in Turkey: an overview. *Int. J. Infect. Dis.* **16**: 228-235.

Zhao, Y., Hanniffy, S., Arce-Gorvel, V., Conde-Alvarez, R., Oh, S., Moriyón, I., Mémet, S., Gorvel, J.-P. (2018): Immunomodulatory properties of *Brucella* melitensis lipopolysaccharide determinants on mouse dendritic cells in vitro and in vivo. *Virulence.* **9**: 465-479.

Zhou, K., Wu, B., Pan, H., Paudyal, N., Jiang, J., Zhang, L., Li, Y., Yue, M. (2020): One Health Approach to Address Zoonotic Brucellosis: A Spatiotemporal Associations Study Between Animals and Humans. *Front. Vet. Sci*. **7**:1-10.

8. ANNEXES

Annex 1: Data recording format for blood sampling from animals

District _____kebele _____Village _____GPS_____ Date_____

NO	lab.No (code)	Species	Sex	Age	Parity	Herd Size	History of Abortion	Still birth	Rfm	Owner's name
1.										
2.										
3.										
4.										
5.										
6.										
7.										
8.										
9.										
10.										

Annex 2: Questionnaire Format Used for Interview Purpose

District _____kebele _____Village _____GPS_____ Date_____

I. Sociodemographic characteristics of the respondents

1. Name of owner (Respondent): _____ Age_____ Sex_____

2. Marital status: Single (1) Married (2) Widowed (3) Divorced (4)

3. Respondent's occupation: Farmer (1) trader (2) employed (3) herder (4) Abattoir worker

4. What is the highest level of education attained by the head of the respondent? (1) No formal Education (2) 1^0 school (3) 2^0 school (4) Vocational school (5) College / University

II. General characteristics of the livestock and livestock managements

5. Herd size ----------

6. Do you keep different species of animals together? a. Yes b. No

7. What types of livestock you reared? a. cattle b. sheep c. Goat d. both/all

8. Where do you get the replacement stock? a. Market b. Raise own replacement c. Both

9. Do you control stray animals from your herd? a. Yes b. No

10. Are there separate parturition maternity pen? a. Yes b. No

11. If your pregnant cows get suffer during birth giving what do you do?

a. I will offer assistance by myself b. Report to veterinarians c. I do nothing

12. For question number 13, if your answer is **a**., do you wear any protective while helping the cow? A. yes B. no.

III. Knowledge and practices of the society about brucellosis

13. Have you ever heard of brucellosis "dhukkuba gatachiisa/ዉርጃ በሽታ/"? a. Yes b. No

14. If yes, from where did you get information about this disease?

A-Relatives/friends B-Veterinarian C-Book D-Media E-I don't know

15. Do you know any disease transmitted from animal to human through handling of infected animals and its products? a. Yes b. No

16. Do you consume a raw milk? a. Yes b. No

17. Do you have a habit of consuming a raw meat? a. Yes b. No

18. Do you thing that diseases can be transmitted from animals to human either through consumption of raw milk or meat? a. yes b. No

19. Do you know any diseases that transmit during handling of delivery or abortion?

a. Yes b. No

20. Have you had cases of abortions in your animals in the past years? a. Yes b. No

21. Which animals aborted frequently? a. cattle b. small ruminants c. both

22. What do think that cause abortion in cows? a. Infectious disease b. non-infectious

23. How do you handle aborted material (the aborted fetus and placental membrane)?

a. Burying b. open dump c. feed to dog d. others___

24. Is there any still birth in your animals so far? a. Yes b. No

25. What do you do to the calving areas after parturition? a. Flushing with water b. disinfecting with detergents c. Both d. I do nothing

26. Do want to have more information about brucellosis? A. Yes b. No

27. Are you volunteer to get tested for this disease? A. yes B. No

Annex 3: Rose Bengal Plate Test Procedures

Sera (control and test sera) and antigen for use will brought to room temperature for half an hour before testing, since active materials straight from the refrigerator react poorly.

1. 30 µl serum is placed and mixed with an equal volume of antigen on a white enamel plate to produce a zone approximately 2 cm in diameter.

2. The antigen and serum are mixed thoroughly using an applicator stick (a stick being used only once)

3. Plate is rocked by hand for about 4 minutes

4. It is examined for agglutination in a good light

Interpretation

0 = no agglutination
+ = barely perceptible
++ = fine agglutination, some clearing +++ = coarse clumping, definite clearing 97.
Those samples identified with no agglutination were recorded as negative those with +, ++, +++ were recorded as positive.

Annex 4: Multispecies i-ELISA procedure

All reagents should equilibrate to room temperature at 21°c ± 5°c before use and homogenized by inversion or vertexing.

1. Add;
    A. 190 µl Dilution Buffer into all wells.
    B.10 µl of the Negative control to A1 and B1
    C.10 µl of the Positive control to C1 and D1
    D. 10 µl of each sample to be tested to the remaining wells.
2. Incubate 45 min (plus or minus 4°c min) at 21°c (plus or minus 5°c).
3. Empty the wells. wash each well 3 times with approximately 300 µl of the wash solution. avoid drying of the wells between washings.

4. Prepare the conjugate by diluting the concentrated10x to 1/10 (short incubator) or to 1/20 (overnight incubator) in dilution Buffer 3

5. Add100 µl of the conjugate 1x to each well.

6. Incubate 30 min plus or minus at 21°c (plus or minus 5°c).

7. Empty the wells. wash each well 3 time with approximately 300 µl of the wash solution.

8. Add 100 µl of the substrate solution to each well.

9. Incubate 15 min plus or minus 2min at 21°c (plus or minus 5°c) in the dark.

10. Add 100 µl of the stop solution to each well in order to stop the reaction.

11. Read and record the O.D.at 450nm

Interpretation For each sample, calculate the (s/p %) as follows using the sample and control values.

Sample positivity percentage (S/p**%) =** $\frac{OD_{sample} - OD_{NTC}}{OD_{PC} - OD_{NC}} \times 100$

Sample with a s/p%;

Less than or equal to110% was considered negative.

Greater than 110% and less than 120 %

Greater than 110% and less than 120 % was considered doubtful.

Greater than or equal to 120% was considered positive

Validation: The test is validated if; the mean value of the positive control OD (ODpc) is greater than 0.350. ODpc >0.350 the ratio of the mean value of the positive and Negative control (ODpc and ODNC) is greater than 3. ODPC/ODNC > 3

Annex 5: Genomic DNA extraction from blood clot procedure.

1. In biosafety cabinet, cut up to 25 mg of blood clot by using sterile forceps and scalpel in sterile Petri dish and place in 1.5 ml screw- cap micro-centrifuge tube.
2. Add 180µl of ATL lysis buffer and 20µl of proteinase K to the tissue
3. Incubate the sample suspension at 56oC for 1-3hr in the water bath. vortexing occasionally to disperse the sample
4.Vortex for 15 min. Briefly spin samples in a mini-centrifuge to remove droplet from under cap. Add 200 µl buffer Alto the sample and mix thoroughly by vortexing. Then add 200 µl ethanol (96-100%), and mix again thoroughly by vortexing.
5. Pippet the mixture (including any precipate) in to the DNeasy Mini spin colomn placed in a 2ml collection tube. centrifuge at 8000rpm for 1 min. Discard flow-through and collection tube.
6. Place the DNeasy Mini spin column in a new 2ml collection tube, add 500µl Buffer AW1, and centrifuge for 1min at 8000rpm. Discard flow-through and collection tube.
7. Place the DNeasy Mini spin column in a new 2ml collection tube, add 500µl Buffer AW2 and centrifuge for 3min at 14,000rpm to dry the DNeasy membrane. Discard flow-through and collection tube.
8. Place the DNeasy Mini spin column in a clean 1.5ml or 2ml microcentrifuge tube and pipet 100 µl Buffer AE directly on to the DNeasy membrane. incubate at room temperature for 1 min. and then centrifuge for 1min at 8000rpm to elute.
9. The eluted DNA is ready to use for PCR

Annex 6: Master Mix preparation for amplification with *IS711* primer

REAGENT/STOCK CONCENTRATION	**FINAL CONCENTRATION**	**µL per REACTION**
Rnase free water	/	3.9 l
IS711 Forward Primer (5-GCT-TGA-AGC-TTG-CGG-ACA-G-3) (20µM)	0.16 µM /25µl	0.2µl
IS711 Reverse Primer(5'-CCT-ACC-GCT-GCG-AAT-3') (20µM)	0.16 µM/25µl	0.2µl
Probe: FAM-AAG-CCA-ACA-CCC-GGC-CAT-TAT-GGT-BHQ-1) (20µM)	0.16 µM/25µl	0.2µl
Exo IPC Mix (10x) IPC primers and probe	1x/25µl	2.5µl
Exo IPC Mix (50x) IPC template DNA	1x/25µl	0.5 µl
Taq man universal PCR master mix(2x)	1x /25µl	12.5µl
VOLUME	20µl	
DNA	5µl	
FINAL REACTION VOLUME	25 µ	

Annex 7: Ethical clearance certificate for human

Oromia Health Bureau
Saarbet (Calcalii) - Finfinnee

የኦሮሚያ ጤና ጥበቃ ቢሮ
ሳርቤት(ጨልጨሊ) - ፊንፊኔ

Lakk/Ref.NO BEFO/[illegible]/126/10/03
Guyyaa/Date 21/7/20[illegible]

Wajjira Eegumsa Fayyaa Godina Shawaa Lixaa tiif
Amboo
Dhimmi: Xalayya Deggersa Kennuu Ilaala.

Akkuma beekamu Biiroon Keenya Ogeyyii, dhabbile akkasumas namoota qorannoo fi Gamaggama jalqabaa fi Xumuraageggessuuf propozaala dhiyeffatan propoozaala isaanii madaaluun akkasumas iddoo biratti ilaalchisani fudhatama argatan (approved) dhiyeffatan, propoozaala isaanii ilaaluudhaan waraqa deggersa ni kenna. Haaluma kanaan qorannoo mata duree ***"SERO-PREVALENCE AND RISK FACTORS ASSOCIATED WITH LIVESTOCK AND HUMAN BRUCELLOSIS IN MALARIA ENDEMIC DISTRICTS OF WEST SHOA ZONE."*** jedhuu irrattii Instiitiyuutiin Fayyaa Hawaasaa Itoopiyaa Biiroo Eegumsa Fayyaa Waliin ta'uun godina keessaan keessaatti qorannoo hojjechuudhaaf Xalayaa deggarsa akka barreessinuuf nuu gafatanii jiru.

Kanaafuu, hojii qorannoo kana irratti deggersa barbaachisa akka gootaniifii isin gaafachaa qorannoon kun qacceffamee ecrga xumuramee booda firii isaa koppii tokko **BEFO** tiif akka galii godhan galagalcha xalayaa kanaan isaan beeksifna. Anis, "**Dr Waaquma Mitikuu**" wayitti qorannoon kun qaaceffame xumuramu firii isaa koppii tokko **BEFO** tiif galii gochuuf mallattoo kootiin ni mirkanessa.

Nagaa Wajjin

Birhaanuu Qanaatee
Hideessaa Garee Qorannoo Fi
Qo'annoo Fayyaa Hawwaasaa

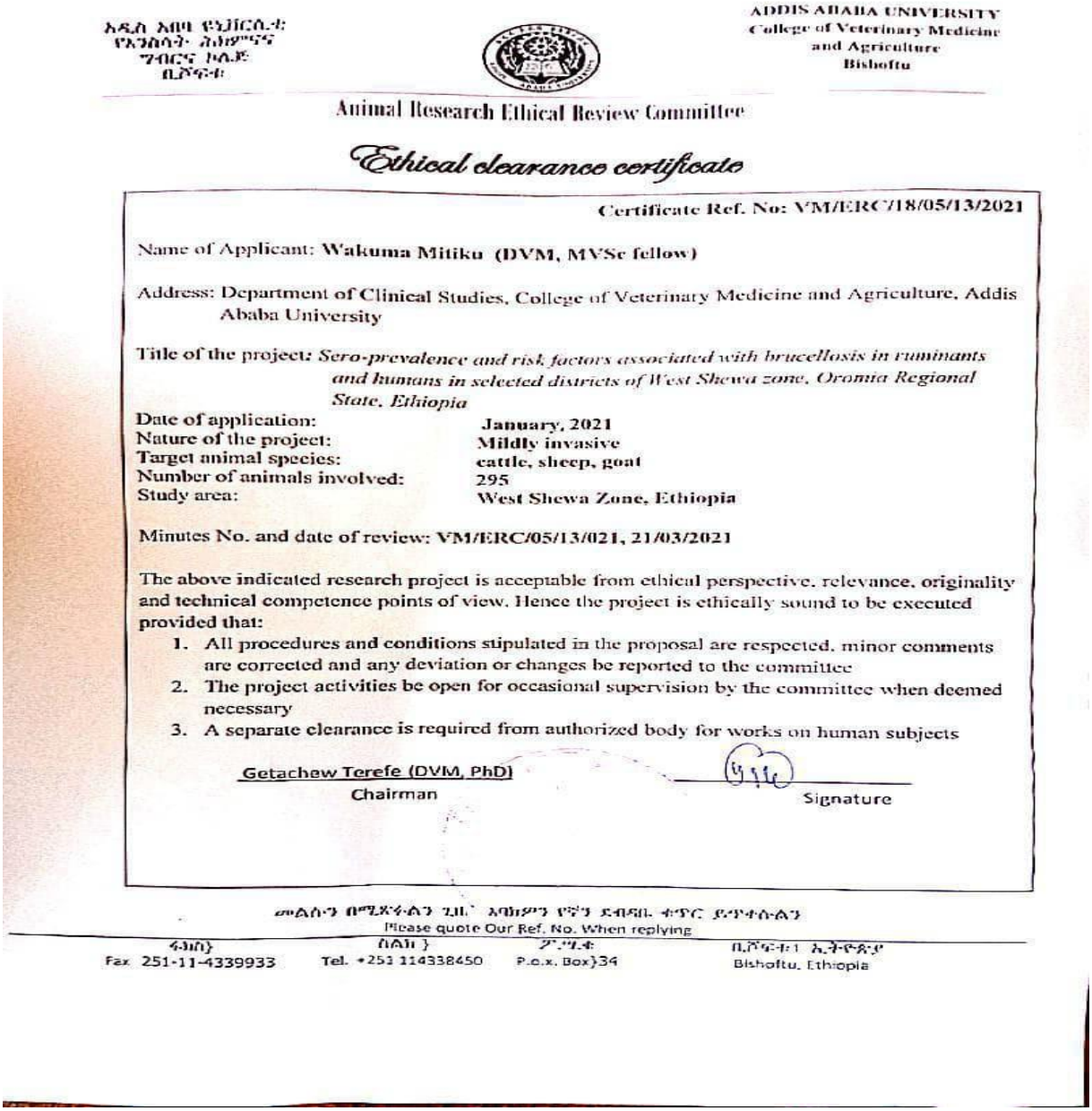
Maqaa: "**Dr Waaquma Mitikuu**"
Mallattoo ________________
Bilbila; **0921174016**
G/G

- "**Dr Waaquma Mitikuu**" *tiif*

B/J

011-371-72-77 befokom2008@gmail.com *or* ohbhead@telecom.net.et Tin NO 000
011-371-72-27 *Oromia Regional Health Bureau* 24341 www.orhb

Annex 8: Ethical clearance certificate for animal



አዲስ አበባ ዩኒቨርሲቲ
የእንስሳት ሕክምናና
ግብርና ኮሌጅ
ቢሾፍቱ

ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/18/05/13/2021

Name of Applicant: **Wakuma Mitiku (DVM, MVSc fellow)**

Address: Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Sero-prevalence and risk factors associated with brucellosis in ruminants and humans in selected districts of West Shewa zone, Oromia Regional State, Ethiopia*

Date of application:	January, 2021
Nature of the project:	Mildly invasive
Target animal species:	cattle, sheep, goat
Number of animals involved:	295
Study area:	West Shewa Zone, Ethiopia

Minutes No. and date of review: VM/ERC/05/13/021, 21/03/2021

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when deemed necessary
3. A separate clearance is required from authorized body for works on human subjects

Getachew Terefe (DVM, PhD)
Chairman

Signature

መልሱን በሚጽፉልን ጊዜ፣ እባክዎን የኛን ደብዳቤ ቁጥር ይጥቀሱልን
Please quote Our Ref. No. When replying

ፋክስ} ስልክ } ፖ.ሣ.ቁ ቢሾፍቱ፣ ኢትዮጵያ
Fax 251-11-4339933 Tel. +251 114338450 P.o.x. Box}34 Bishoftu, Ethiopia