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**BRUCELLOSIS IN CATTLE, CAMEL AND HUMAN: SEROPREVALENCE AND
ASSOCIATED RISK FACTORS IN AMIBARA DISTRICT OF AFAR REGION,
ETHIOPIA**

MVSc THESIS



**ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND
AGRICULTURE, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND
VETERINARY PUBLIC HEALTH**

BY

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JUNE 2020

BISHOFTU, ETHIOPIA

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ASSOCIATED RISK FACTORS IN AMIBARA DISTRICT OF AFAR REGION,
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A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirement for the degree of Master of Veterinary Science in Veterinary Public Health.

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DEDICATION

This piece of work is dedicated to the victims of covid-19 pandemic in the world specially those who lost their life.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirement for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ALIPB	Aklilu Lemma Institute of Pathobiology
AMOS	Abortus, Melitensis, Ovis, Suis
BSL	Biosafety Level 2
CDC	Center for Disease Control
CFT	Complement Fixation Test
CFSPH	The Center for Food Security and Public health
CI	Confidence Interval
CSA	Central Statistics Agency
CVMA	College of Veterinary Medicine & Agriculture
DNA	Deoxyribonucleic Acid
ELISA	Enzyme linked Immuno Sorbent Assay
FAO	Food and Agricultural Organization
GC	Guanine-Cytosine
GIS	Geographic Information System
GTPase	Guanosine Triphosphatase
HIV	Human Immuno Deficiency Virus
IGAD	Intergovernmental Authority for Development
LFA	Lateral Flow Assay
LPS	Lipopolysaccharide
Mbp	Mega base pair
MLVA	Multiple Locus Variable Number random repeat analysis
MOA	Ministry of Agriculture
NAHDIC	National Animal Health Diagnostic and Investigation Center
NVI	National Veterinary Institute
OIE	Office of International Des Epizootics
Omp	Outer Membrane Protein

LIST OF ABBREVIATIONS(Continued).

OR	Odds Ratio
OWC	Old World Camel
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PG	Protective Gloves
QGIS	Quantum Geographic Information System
RBPT	Rose Bengal plate test
RER	Rough Endoplasmic Reticulum
RFM	Retained Fetal Membrane
Rho	Ras homologous
R-LPS	Rough Lipopolysaccharides
S-LPS	Smooth Lipopolysaccharides
SSA	Sub-Saharan Africa
SAT	Serum Agglutination Test
USSR	United Soviet Socialist Republic
WARC	Werer Agricultural Research Center

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ABSTRACT

A cross-sectional study was conducted to determine the seroprevalence of brucellosis in camels, cattle and human and its associated risk factors in Amibara district of Afar region from October 2019 to May 2020. A total of 250 camels, 181 cattle and 120 human sera were collected. Sera were screened using Rose Bengal Plate Test (RBPT) and samples positive for RBPT were further confirmed by Complement Fixation Test (CFT). Risk factors associated with brucellosis in livestock and human were also assessed by structured questionnaire. Association of risk factors with brucellosis were analyzed using chi-square (fisher exact test) and firth's bias reduced logistic regression model. The result indicated that, the overall seroprevalence of brucellosis was 7.6% (95% CI:0.04-0.11) by RBPT and 3.2 % (95% CI: 0.0163-0.062) confirmed by CFT in camel and 10.5% (95% CI:0.068-0.158) by RBPT and 2.2% (95% CI:0.0086-0.0553) confirmed by CFT in cattle respectively. In human the overall seroprevalence of brucellosis was 10% (95% CI:0.058-0.167) by RBPT and 3.33% (0.013-0.082) as confirmed by CFT. The risk factors analysis indicated that, age ($\chi^2=10.7$; $p=0.004$), body condition ($\chi^2=10.26$, $p=0.0022$), number of parity ($\chi^2=11.2$, $p=0.0008$) and abortion history ($\chi^2=29.96$; $p=0.004$) were significantly associated with seropositivity of camel brucellosis. But, kebeles, sex, herd size and history of placental retention were not significantly associated with brucellosis seropositivity ($P>0.05$). Based on multivariable firth's bias reduced logistic regression analysis, only camels with history of abortion (OR=49.6, $P=0.002$, 95% CI=2.148-6.34) were significantly associated with brucellosis. In cattle, age ($\chi^2=6.77$, $p=0.021$), number of parity ($\chi^2=9.433$, $p=0.004$), abortion history ($\chi^2=16$, $p=0.002$) and placental retention ($\chi^2=19.1$, $p=0.003$) were significantly associated with brucellosis seropositivity. Multivariable firth's bias reduced logistic regression analysis indicated that, only number of parity (OR=12.2, $P=0.0012$, 95% CI=7.47-3.17) and history of abortion (OR=41.14, $P=0.003$, 95% CI=1.16-8.69) were significantly associated with Brucella infection. In human, occupation ($\chi^2=6.925$, $P=0.028$), handling of dystocia case, usage of protective gloves and sheltering of animals showed significant association with Brucella seropositivity. Likewise, multivariable firth's bias reduced logistic regression was computed and only daily based temporary occupation type showed significant association with brucellosis seropositivity (OR=18.85, $P=0.029^*$, 95%CI=1.324-2730.32). The survey results also showed that, from a total of 120 human participant interviewed, 91.7% (110/120) of them drink fresh raw milk on a regularly basis where by 2.78% of them owned sero-positive animals and 3.33% of them tested positive for the disease. The results of the present study indicated that, brucellosis is a common health problem in camel, cattle and human in Amibara district of Afar region and it was found to be associated with raw milk consumption and close contact with the animals having history abortion. Therefore, controlling the risk factors, establishing brucella diagnostic service in human clinics and hospitals, continuous social training with feedback assessments and overall implementing of One Health approach framework to attain optimal health for people and domestic animals in area are recommended to safeguard the health of society.

Key Words: *Amibara, Afar, Abortion, Brucellosis, Camel, Cattle, Human, Seroprevalence.*

1. INTRODUCTION

Brucellosis is an important zoonotic disease caused by infection with bacteria of the genus *Brucella* affecting different mammalian species including man (CFSPH, 2018). It is an economically important infectious bacterial disease which severely hinders livestock productivity (Yasmin and Lone, 2015). The disease affects mainly domestic animals such as cattle, sheep, goats, pigs and dogs as well as human (Lopes *et al.*, 2010). Brucellosis has also been documented in wildlife and marine animals (Godfroid, 2002) whereas domestic carnivorous animals may acquire infection by consuming contaminated fetuses, meat, placentae or milk (Woldemeskel, 2013). The disease came to prominence during the 1850's Crimean war in Malta, which is claimed by thousands of British soldiers (Madut *et al.*, 2019).

Fifty years ago, the *Brucella* genus contained only *B. abortus*, *B. melitensis*, and *B. suis*, the 3 classic species of brucellae where the taxonomy placed them in the family Brucellaceae, with other bacterial genera such as *Bordetella* and *Pasteurella* (Olsen and Palmer, 2014). Currently 11 *Brucella* species are recognized with high genetic similarity although each has different host preference (Mathew *et al.*, 2015). However, the species of *Brucella* bacteria with great zoonotic and economic importance are *B. abortus*, *B. melitensis*, and *B. suis* (Mathew *et al.*, 2015). All these *Brucella* organisms can enter the body of animals through inhalation, ingestion and mucous membrane or broken skin (Corbel, 2006).

Brucellosis has a worldwide distribution and is one of the most important zoonoses in Africa, Asia, Mediterranean region and Middle East (De Massis *et al.*, 2019) where Africa is one of the endemic areas. In several areas of East African region; it significantly reduces animal productivity through abortion and weak offspring's; causing a major threat in national and international livestock trade (Ntirandekura *et al.*, 2018a). In Ethiopia, brucellosis is found to be one of the endemic disease of livestock associated with reproductive problem all across the country. Significant loss of productivity through abortion, late first calving age, long calving interval time, low herd fertility and comparatively low milk production were reported in livestock (Abebe *et al.*, 2017 ; Megersa *et al.*, 2011).

According to Muma *et al.*, (2007) and Schelling *et al.*, (2003) report, cows infected with *Brucella* are three to four times more likely to abort than unexposed cows. Additionally, this disease posed a barrier to export and import of animals, constraining livestock trade and is an impediment to free animal movement(Yilma *et al.*,2016). In human, brucellosis is a debilitating disease that lacks pathognomonic symptoms(Ducrotoy *et al.*, 2017), representing a major public health hazard, which affects social wellbeing and stability in many countries(Njeru *et al.*, 2016). Brucellosis is mainly an occupational zoonotic diseases and there is an increased incidence of human brucellosis in persons who are engaged in certain professions such as veterinarians, slaughterhouse employees, dairy farmers, pastoralists, livestock handlers, and laboratory personnel (Esmaeili *et al.*, 2016 ; Kiambi, 2014).

Food and Agricultural Organization (FAO), World Health Organization (WHO) and World health organization for animals (OIE) consider brucellosis the most highly spread zoonosis in the world with annual occurrence of more than 500,000 human cases (Hull and Schumaker, 2018 ; OIE, 2004). The disease spread when people consume unpasteurized contaminated milk, raw liver(rare) and contact with infected tissues and discharge(Bosilkovski, 2015). Based on the nature of the disease and ease of transmission, the pastoral society of Africa and central Asia are at great risk due to their habit of consumption of raw milk, close physical contact and assistance in calf delivery, cleaning newborn, suckling and carrying the young calf from field to nomadic house(Abbas and Agab, 2002). However, because of the difficulty to access pastoral communities, the occurrence and the control of brucellosis is poorly understood both in humans and animals in the pastoral settings of the Sub-Saharan Africa where the burden of the disease could be high(McDermott and Arimi, 2002).

In Ethiopia, the majority of Afar, Somali and Borana peoples are pastoral communities who mainly depends on livestock and where the livestock population approximately account for 42% of the national figure (Yosef *et al.*, 2013). In these pastoral regions, brucellosis in animals and humans has been reported by different authors where the prevalence was quite varying and largely confined to serological surveys (Yohannes *et al.*, 2013). According to Zerfu *et al.*, (2018) , the incidence rates of brucellosis in humans of pastoral and sedentary system origins were estimated at 160 and 28 per 100 000 persons in a year, respectively.

In cattle's; seroprevalence was predominantly reported by (Berhe *et al.*, 2007; Mekonnen *et al.*, 2010) while the status of the disease in camels(Teshome *et al.*, 2003) was not well documented due to the difficulty in gathering information and to their mobility. Establishment of adequate control programs against brucellosis in a population depends on the presumptive diagnosis of the infection and requires knowledge of its epidemiology in different animal species. Even though, isolation and identification of brucella bacteria is the gold standard diagnosis of brucellosis, limited research work was conducted for the isolation and identification of *Brucella* species from cattle and camel in Ethiopia which is a clearly visible research gap.

So far, studies made on seroprevalence of cattle, camel and human brucellosis were not exhaustively studied and there is no clear understanding of the geographic pattern of brucellosis. Additionally, the public health implication of brucellosis in the pastoral areas of Afar region has not been extensively studied and evaluated by diagnostic techniques rather than relying on survey works (Zerfu *et al.*, 2018). Hence, the availability of recent finding could aid in instituting proper control and prevention measures against this disease for animal owners and communities of the areas at large. Therefore, the objectives of this study were

1.1. General Objective

- To determine seroprevalence of brucellosis in cattle and camels and exposed individuals for assessment of its public health importance in Amibara district of Afar Region; Ethiopia

1.2. Specific Objectives

- To determine seroprevalence of brucellosis in cattle and camels in Amibara district
- To determine the seroprevalence of brucellosis in livestock owners (pastoralists) in the study area
- To investigate potential risk factors of brucellosis in cattle and camel and its zoonotic risk factors in human in Amibara district of Afar Region
- To assess the knowledge, attitude and practice of the community about zoonotic brucellosis in Amibara district of Afar region; Ethiopia

2. LITERATURE REVIEW

2.1. Etiology

Bacteria of the genus *Brucella* are the causative agents of brucellosis affecting a wide range of warm-blooded land and marine vertebrates (Atluri *et al.*, 2011). To fully understand the current nomenclature, taxonomy, overall structure, and molecular diversity of the genus *Brucella*, knowledge about its creation and history is a prerequisite (Scholz and Vergnaud, 2013). The genus *Brucella* was established by Meyer and Shaw in 1920, with *B. abortus* mainly biovar 1 infecting cattle (Scholz and Vergnaud, 2013). However, *B. melitensis* and rarely *B. suis* can also establish themselves in cattle with subclinical manifestation (Neta *et al.*, 2010). Camels can be infected by either of the main species of the genus *Brucella* (*B. abortus* and *B. melitensis*) (Abbas and Agab, 2002).

Worldwide, the majority of human cases are attributed to *B. melitensis* as the most pathogenic and virulent species for humans followed by *B. suis*, while *B. abortus* is considered to induce mildest type of brucellosis (Benkirane, 2006 ; Pappas *et al.*, 2006.; Galinska and Zagórski, 2013). *Brucella* are Gram-negative facultative intracellular cocco-bacilli that are none capsulated, non-spore forming and non-motile belonging to the alpha-2 subdivision of the proteobacteria (Seleem *et al.*, 2010). Currently; eleven *Brucella* species are recognized, including the better known six classical species comprised of *B. abortus* (biovars 1-6, and 9), *B. melitensis*, (biovars 1-3), *B. suis* (biovars 1-5), *B. ovis*, *B. canis* and *B. neotomae*. The more recently, identified new members of *brucella* species include; *B. ceti* and *B. pinnipedialis*, *B. microti* (voles) and *B. inopinata* (Godfroid *et al.*, 2011).

Brucella organisms grow slowly, but can be enhanced by using enriched media whereas certain strains needs 5% to 10% of carbon dioxide for growth The addition of 2–5% bovine or equine serum is necessary for the growth of strains such as *B. abortus* biovar 2, and many laboratories systematically add serum to basal media, such as blood agar base (Oxoid) or Columbia agar with excellent results (OIE., 2008).

Concerned with resistance and survival of Brucella organisms, they may be killed at a temperature of sixty degrees Celsius (60 °C) for ten minutes, but dense suspensions, such as laboratory cultures, require more drastic heat treatment to ensure their inactivation(Quinn., 1994). Additionally; most disinfectants active against other gram-negative bacteria can kill them; even though they can survive freezing and thawing. Pasteurization can also effectively kill Brucella in milk(Alton *et al.*, 1988). The brucella bacterium is of 0.5-0.7µm in diameter and 0.6-1.5µm in length. They are oxidase, catalase and urease positive. Although Brucella species are described as non-motile, they carry all the genes except the chemotactic system necessary to assemble a functional flagellum(Fretin *et al.*, 2005). Brucella species are distinguished based host preference and phenotypic characteristics(Seleem *et al.*, 2010;O’Callaghan and Whatmore, 2011).

However, host preference is not absolute and most of the species of Brucella bacteria have been isolated in multiple different hosts(Potter, 2013). For instance, some Brucella species like *B. abortus*, *B. melitensis*, *B. suis* and *B. canis* can affect a range of hosts in addition to their natural hosts resulting hazards on the health of animals including humans(Yasmin and Lone, 2015). Accordingly; *B. abortus* and *B. melitensis* are the major causative agents of brucellosis in camel and cattle even though camels are not found to be their primary host(Foster *et al.*, 2018). Due to this, infected countries are challenged and have been under difficulties to overcome or control brucellosis effectively(Liu, 2014). Generally; *B. abortus*, *B. melitensis*, and *B. suis*, are the main pathogenic species worldwide and are responsible for cattle, caprine/ovine and swine brucellosis respectively. These 3 Brucella species cause abortion storm which result in huge economic losses(Ntirandekura *et al.*, 2018a).

Table 1: *Brucella* species and biovars, preferential hosts and pathogenicity for humans

Species	Biovars	Colony morphology	Preferential host(s)	Pathogenicity in humans
<i>B.melitensis</i>	1-3	Smooth	Goat, sheep	High
<i>B.abortus</i>	1-6, 9	Smooth	Cattle	High
<i>B.suis</i>	1, 3	Smooth	Pig	High
	2	Smooth	Wild boar, hare	Low
	4	Smooth	Reindeer, caribou	High
	5	Smooth	Rodent	No
	-	Smooth	Desert rat	Moderate
<i>B.neotomae</i>	-	Smooth	Desert rat	Moderate
<i>B.ovis</i>	-	Rough	Ram	No
<i>B. canis</i>	-	Rough	Dog	moderate
<i>B.pinnipedialis</i>	-	Smooth	Seal	? ⁺
<i>B. ceti</i>	-	Smooth	Cetacean	?
<i>B.microti</i>	-	Smooth	Soil, vole, fox	?
<i>B. inopinata</i>	-	Smooth	Human	?

?⁺Although some human cases have been described, the actual pathogenicity remains unknown.

Adapted from:(Godfroid *et al.*, 2010).

2.1.1. Genome and morphology of *Brucella*

Due to its great economic and zoonotic importance, it is useful to identify field isolates of *Brucella* not only at their species level but also their genotypes. This enables the detection of hidden foci of *Brucella* and to tract the sources of infection in the population. Additionally; there can be a genomic divergency between a single *Brucella* spp isolated from different host(El-Sayed and Awad, 2018). However; the genomes sequenced from genus *Brucella* are known to be very similar in terms of both base composition and genome size. All sequenced species have a GC content of approximately 57%, and most genomes consist of approximately 3.3 Mbp divided on two chromosomes. Housekeeping genes, including those involved in DNA replication, transcription, translation, core metabolism, and cell wall biosynthesis are distributed on both chromosomes(O'Callaghan and Whatmore, 2011). *B. melitensis* is the first *Brucella* species to be sequenced 16M (biovar 1) followed closely by *B. suis* (biovar 1)(Bohlin *et al.*, 2010). Meanwhile, none of the sequenced members of the *Brucella* genus have any plasmids reported.

Comparison of *B. suis* with *B. melitensis* indicates that the majority (>90%) of *B. suis* and *B. melitensis* genes share 98-100% identity at the nucleotide level. The more variable genes (<95% identity) consist primarily of hypothetical genes, urease component, and probable surface-exposed genes. These more variable genes may contribute to the differences in pathogenicity or host preference between these two organisms(Liu, 2014)

Brucella are very small faintly stained coccoid rods, with a microscopic appearance of fine sand. Primary culture of brucella reveals punctate, non-pigmented, and non-hemolytic colonies. Colonies of smooth brucella strain are raised, convex, circular, translucent and 0.5-1 mm in diameter. The colony morphology of brucella may become less convex and more opaque with a dull, dry, yellowish, white granular appearance which is caused by dissociation of brucella from smooth to rough forms(Liu, 2015).

The morphology of the Brucella bacterial colonies is associated with the presence of lipopolysaccharides (LPS) in the external membrane of the bacterium. The S-LPS phenotype is found in most Brucella species, and only *B. canis* and *B. ovis* possess the R-LPS(Mancilla, 2016). Optimal temperature for culture is 37°C, but the organism can grow under temperatures ranging from 20°C to 40°C, whereas optimal pH ranges from 6.6 to 7.4. Typical colonies appear after 2 to 30 days of incubation, but a culture can only be considered negative when there are no colonies after 2 to 3 weeks of incubation. False negative results should be considered in the absence of bacterial growth since the sensitivity of culture is low(Poester *et al.*, 2010).

2.2. Epidemiology of Brucellosis

2.2.1. Global distribution of livestock brucellosis

Brucellosis is a worldwide bacterial disease affecting both animals and humans which subsequently causes serious human health hazards and economic loss. The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging (Seleem *et al.*, 2010). It was reported in camels as early as in 1931 by Solonitsiun in Russia. Since then, serological evidence of brucellosis has been reported from the most important camel keeping countries (Bayasgalan *et al.*, 2018). Camel brucellosis is a wide spread disease in camel rearing regions of the world such as middle East and the Arabian Gulf, parts of Africa, and Latin America with the exception of Australia, Canada, New Zealand, England, Japan (Potter, 2013; Robinson, 2003; Wernery, 2014)

In cattle, the disease occurs worldwide where cattle rearing is practiced, except countries such as Australia, Canada, Cyprus, Denmark, Finland, Netherlands, New Zealand, Norway, Sweden and the United Kingdom which has eradicated. This is based on the absence of any reported cases for at least five years (Seleem *et al.*, 2010). However, the Mediterranean Countries of Europe, Africa, Far East countries, India, Central Asia, Mexico, Central and South America are still not brucellosis free. Although in most countries brucellosis is a nationally notifiable disease and reportable to the local health authority; it is under reported and official numbers constitute only a fraction of true incidence of the disease (Ashley Robinson, 2003).

In Brazil, cattle brucellosis due to *B. abortus* is the most prevalent Brucella infection, followed by the one caused by *B. suis* in pigs. The economic impact of cattle brucellosis in Brazil has been estimated at 32 million dollars annually (Poester *et al.*, 2002). In Africa, evidence obtained throughout the years shows that, brucellosis is a widespread problem despite under reporting and the scarcity of epidemiologically valid data (Ducrottoy *et al.*, 2017). In sub-Saharan Africa, the occurrence of camel brucellosis (either prevalence or incidence) is not well documented and reports submitted to the World Organization for Animal Health (OIE) are largely confined to serological surveys, which are mainly conducted for cattle, sheep, goats and less for camel.

With large pastoral communities, and the demand for meat and livestock products is simulated to double by 2050, brucellosis is expected to pose a major threat in Sub Saharan Africa (Racloz *et al.*, 2013). According to Ekere *et al.*, (2018), the disease has a cosmopolitan distribution, and affects economically important domestic animals including wild life. Persistent case of brucellosis was observed in most African countries like Tanzania, Nigeria, Uganda, Kenya, Zimbabwe and Somalia reporting brucellosis in humans and domestic animals (Racloz *et al.*, 2013). In East Africa, brucellosis is reported in most member countries of IGAD and endemic with high economic loss and zoonoses (Zewdie, 2018). In countries with more extensive form of husbandry practice, such as Chad and Ethiopia, the seroprevalence of camel brucellosis is 3.8% and 5.5% respectively (Wernery, 2014). In Nigeria, the disease has been reported from nearly all camel producing areas (Salisu *et al.*, 2018).

2.2.2. Distribution of livestock brucellosis in Ethiopia

In Ethiopia, there is no documented information on how and when brucellosis was introduced and established. But the disease was reported for the first time in 1970s (Meyer, 1980) and has been noted as one of the important livestock diseases in the country. Since then, several studies have been carried out to determine its prevalence in different parts of the country (Berhe *et al.*, 2007 ; Jergefa *et al.*, 2009). Wide range of livestock production; different management systems, multiple livestock species per holding, stock density and social organizations to handle livestock may account for the widespread occurrence; maintenance and transmission of cattle brucellosis (Megersa *et al.*, 2011). Though, about 40% of livestock populations are kept under the pastoral lowland of Ethiopia; most brucellosis surveys were focused on several institutionally owned commercial dairy farms which are mostly situated in and around Addis Ababa and in some regional towns (Yohannes *et al.*, 2013). However, many studies have been carried out recently to determine the status of cattle brucellosis which showed wide spread occurrence of the disease in different livestock production systems (Asgedom *et al.*, 2016). For instance, number of articles have been published reporting individual seroprevalence ranging from 1.1% to 22.6% in intensive management systems. and 0.1-15.2% in extensive management system (Tesfaye *et al.*, 2011). Nevertheless, most reports made so far have either limited geographic coverage or are relatively confined to a single agro-ecology (Asmare *et al.*, 2013).

Table 2: Seroprevalence of cattle brucellosis in some parts of Ethiopia

Location	No. examined	Prevalence rate (%)		References
		RBPT	CFT	
Tigray	816	3.3%	3.19%	(Berhe <i>et al.</i> , 2007)
East Wollega	406	2.96%	1.97%	(Yohannes <i>et al.</i> , 2012)
Addis Ababa	1202	-	1.5%	(Tsfaye <i>et al.</i> , 2011)
East Showa	300	3.3%	2%	(Alemu <i>et al.</i> , 2014)
North Gondar	780	1.28%	0.5%	(Tedele <i>et al.</i> , 2010)
Ambo	169	0.2%	0%	(Bashitu <i>et al.</i> , 2015)
Debrebirhan	246	0.7%	0.2%	(Bashitu <i>et al.</i> , 2015)
Central Ethiopia	1136	12.5%	11%	(Kebede <i>et al.</i> , 2008)
Western Ethiopia	1152	1.2%	79%	(Adugna <i>et al.</i> , 2013)
Arsi Zone	370	0.05%	0.05%	(Degefa <i>et al.</i> , 2011)

In camel, brucellosis has been reported from pastoral areas, where the prevalence was quite varying ranging between 0.73- 11.9% for RBPT and 0.53-9.6% for CFT(Yilma *et al.*, 2016). In the South Omo zone of Ethiopia; 4.2% of prevalence rate was reported in camel. On the other hand; Woldegebriel reported a prevalence rate of 7.6% in the Amibara district of the Afar region (Melaku and Tessema, 2013). This variation in seroprevalence of camel brucellosis is attributed to the difference in animal husbandry and management systems practiced by pastoral society (Awole *et al.*, 2002). Compared with other neighboring African countries and middle East, lower seroprevalence of camel brucellosis was recorded by(Tilahun *et al.*, 2013) in pastoral areas of Ethiopia.

Table 3: Seroprevalence of camel brucellosis in Ethiopia

District	No. of examined	Prevalence rate (%)		Reference
		RBPT	CFT	
	768	11.9%	7.6%	(Zewold and Wereta, 2012)
Afar	460	5.4%		(Bekele <i>et al.</i> , 2013)
	1152	5%	4.7%	(Hadush <i>et al.</i> , 2013)
Somali, Afar& Oromia	1442	5.7%	4.2%	(Teshome <i>et al.</i> , 2003)
Southern Ethiopia	1830		0.9%	(Gumi, 2013)
Akaki	201	6.5%	4.5%	(Abebe <i>et al.</i> , 2017)
Jigjiga & Babile	822	2.43%		(Tilahun <i>et al.</i> , 2013)
Dire dawa	646	2%	1.5%	(Warsame <i>et al.</i> , 2012)
Borana	756		2.2%	(Megersa <i>et al.</i> , 2011)
	1073		1.8%	(Megersa <i>et al.</i> , 2012)
Bale & Borana	1500		0.53%	(Tesfaye <i>et al.</i> , 2014)
Yabello	384	3.6%	3.1%	(Admasu and Kaynata,2017)

Notes: N₀. A= (Number of animals examined)

2.3. Risk factors

2.3.1. Animal risk factors (Age and Sex)

Age has been referred to as one of the intrinsic factors associated with brucellosis in animals. According to Bekele *et al.*, (2011) report, brucellosis has traditionally been considered as a disease of adult animals since susceptibility increases after sexual maturity and pregnancy. This is due to the fact that, *Brucella spp.* presents tropism to the reproductive tract due to the production of erythritol sugar in the fetal tissues (Coelho *et al.*, 2015). Long time contact with infected animals or with the environment also contributes to the higher prevalence of brucellosis in adults animals which is significantly seen in those herd without culling of positive animals(Megersa *et al.*, 2012).

The influence of sex in the prevalence of brucellosis has been studied in domestic and wild animals (Muñoz *et al.*, 2010). In camels, females are more susceptible to brucellosis than male. This relatively higher susceptibility of female camels could be due to the fact that they have more physiological stresses than the males (Salisu *et al.*, 2018). According to Hirsh and Zee, (1999), male animals are less susceptible to *Brucella* infection due to the absence of erythritol sugar which is found in the uterus. Also female camels and cattle's are kept longer in herds for breeding purposes than males which are fattened and sold off except for a few that are kept to service the females, for haulage, transport, draught power and other purposes (Salisu *et al.*, 2018).

2.3.2. Environmental and Management risk factors

Brucellosis can occur in any season of a year. However, February to July is the season of the year when peak epidemics of brucellosis occurs and is closely related to the months associated with delivery and abortion in animals (Asnake *et al.*, 2017). Uncontrolled trade of clinically inconspicuous animals can also leads to high individual animal and herd prevalence whereas habitat, herd size, cohabitation with other ruminants, and contact with other infected animals, leads to an inter-camel cycle of the disease which is the same for other animals (Ghanem *et al.*, 2009).

Further risk factors are the increase in species composition at household level, and the wet season. Due to this, camels appear to become infected via spill-over from small ruminants and cattle. This observation is supported by the fact that all *Brucella spp.* and biovars can infect multiple host animals. According to Musa *et al.*, (2008) report, the higher prevalence of brucellosis (23.8%) from camel kept mixed with ruminant species was recorded. Other factors aggravating the occurrence of brucellosis includes uncontrolled animal movement, migrations of pastoralists in search of pasture and water, purchase of infected cattle from livestock market for replacement or upgrading, anarchic development of urban livestock breeding and nature of the animal production system, inadequate sanitary measures, demographic factors, regulatory issues, climate, deforestation and wildlife interaction (Awah-Ndukum *et al.*, 2018).

2.3.3. Pathogen risk factors

B. abortus and *B. melitensis* are the main etiological agents of camel and cattle brucellosis and responsible for an economically important cause of abortions. *B. abortus* also affects other species such as bison, buffalo or elks representing an important risk for the maintenance of the agent in the animal population with special importance in areas where wildlife and domestic animals live together. Moreover, infections in wildlife can hinder eradication efforts in domestic animals. *B. abortus* is still a human pathogen and outbreaks arise from contact with infected animals and ingestion of contaminated dairy products represent an important risk of infection (Coelho *et al.*, 2015).

2.4. Transmission of Brucellosis

The primary shedding routes of *Brucella* organisms remain uterine fluids and placenta expelled from infected animals. Due to this, both domestic and wild animals can contract brucellosis through direct contact with infected animals and their excreta. Many placental mammals, including herbivores, participate in placentophagy, with camel as a noted exception, which may contribute to the spread of *Brucella* bacteria. Although parturition in camels is generally occurred in a laying or standing position without extra help, they may deliver or abort on the pasture and the aborted material may spread over a wide area of the pasture by stray dogs and foxes. This play an important role for the transmission of the disease to other health animals (Gwida *et al.*, 2012).

On the other hand, a close contact between infected and susceptible animals in a herd promotes the spread of diseases. The camels and cattle's share the same watering points and pastures with other livestock and so it is not surprising to find a higher incidence of the disease among these animals (Teshome *et al.*, 2003). Many researchers disclosed that, survival of the organisms in the environment is enhanced by cool temperatures and humidity which allows maintenance of the bacterial in the environment for fairly long period of time where many susceptible animals can be exposed (Wernery, 2014). Additionally, it was proven that two dromedaries in a *Brucella* negative dromedary herd were infected with *B. melitensis* through contaminated dust particles from aborted camel fetuses 500m apart, indicating that organisms can also survive in a hot desert

environment(Wernery, 2014). Generally, animals become infected through feed, water, colostrum, venereal transmission, contaminated milk and, especially, by licking or sniffing at placentas and aborted fetuses. In human, brucellosis is transmitted by contact with infected animal, consumption of unpasteurized dairy products and undercooked meat, drinking camel urine(Salisu *et al.*, 2018) including aerosol transmission (Minogue *et al.*, 2014). For instance, consumption of traditional delicacies such as raw liver can cause human infection(Gwida *et al.*, 2010).



Figure 1: Unhygienic milking and drinking of raw camel milk.

Source: (Own photo., 2018 ; MOA., 2017)

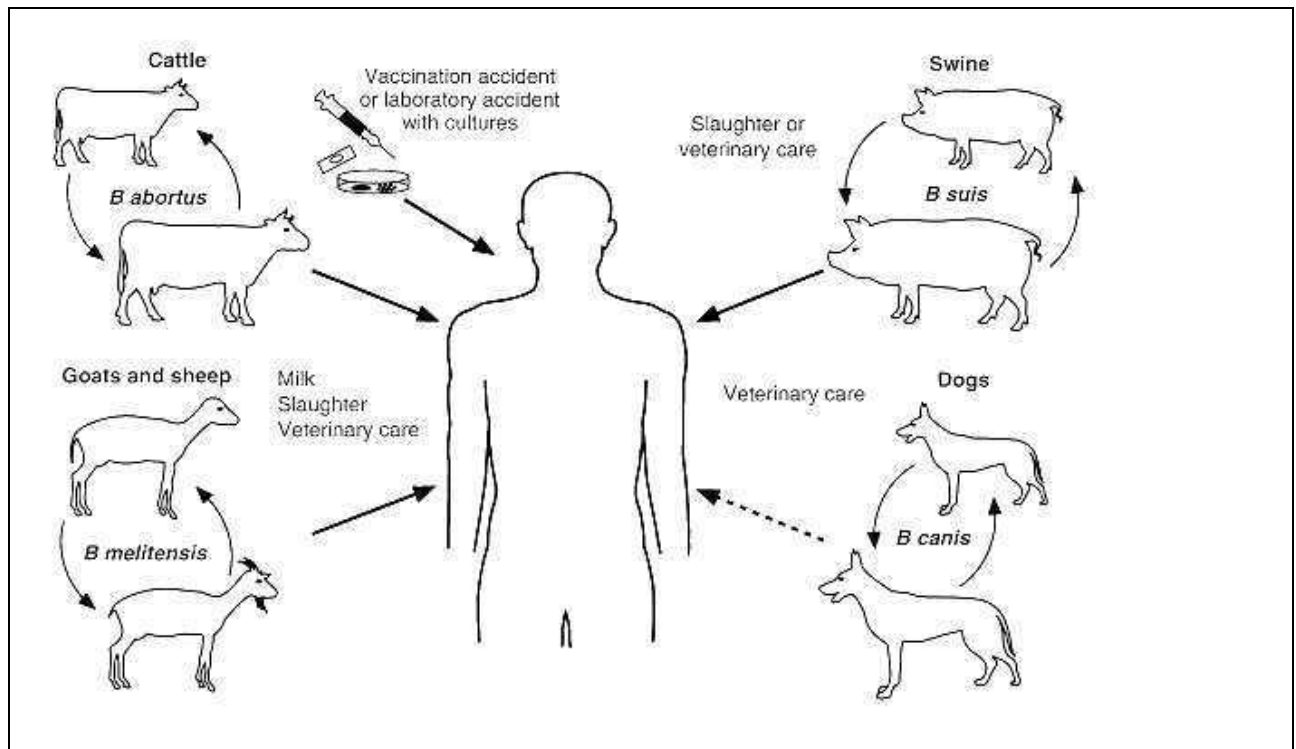


Figure 2: Transmission route of brucellosis among animals and to humans.

Source:(Alton and Forsyth., 1996)

2.5. Pathogenesis and clinical sign of Brucellosis

2.5.1. Pathogenesis

Brucella organisms are pathogens that ultimate goal is to propagate in their preferred niche, the cell. The ability of *Brucella spp.* to cause disease requires a few critical steps during infection. Although the mechanisms that allow host cell invasion by *Brucella spp.* are not completely clear, internalization of Brucella into host cells requires cytoskeletal changes. *Brucella spp.* can invade epithelial cells of the host, allowing infection through mucosal surfaces: M cells in the intestine have been identified as a portal of entry for *Brucella spp* (Poester *et al.*, 2013). Upon cell contact, the bacteria are internalized via receptor molecules by activating small GTPases of the Rho subfamily and by a moderate recruitment of actin filaments(Gorvel and Moreno, 2002).

Interestingly, invasion through the digestive tract does not elicit any inflammatory response from the host and therefore, *Brucella spp.* invade silently or unnoticed by the innate immune system of the host(Barquero-Calvo *et al.*, 2007). Once *Brucella spp.* have invaded, usually through the digestive or respiratory tract, they move to regional lymph nodes and are capable of surviving intracellularly within phagocytic or non-phagocytic host cells with the help of enzyme called cytochrome oxidase(Seleem *et al.*, 2008). On the other hand, acidification of the Brucella containing vacuole during early steps of infection is also required for intracellular survival since acidified environment induces changes in the profile of bacterial gene expression favoring intracellular survival(Neta *et al.*, 2010).

So, the pathogenicity of Brucella is due to its ability to adapt to the environmental conditions encountered in its intracellular replicative niche including low levels of nutrients and oxygen, acidic pH and reactive oxygen intermediates(Seleem *et al.*, 2008). Inside the cells, Brucella has the ability to interfere with intracellular trafficking, preventing fusion of the Brucella containing microphages(phagosomes) with lysosome markers, and directing the vacuole toward a compartment that has rough endoplasmic reticulum (RER), which is highly permissive to intracellular replication of Brucella (Marchesini *et al.*, 2011).

Then, *Brucella spp* disseminate throughout the body and induces suppression of the transcription of pro-inflammatory mediators in trophoblastic cells at very early stages of infection in female(Neta *et al.*, 2008). After an initial suppression of pro-inflammatory transcripts, Brucella bacteria induces expression of pro-inflammatory chemokines which finally results in abortion in female animals(Neta *et al.*, 2008). This rapid multiplication of Brucella organisms results in the development of severe ulcerative placentitis endometritis of the inter-cotyledonary spaces with development of yellowish gelatinous fluid in pregnant animals(Gabli *et al.*, 2015). Even though the exact mechanism under which abortion occur is not known, but it is believed to be due to the interference with fetal circulation as a result of placentitis, or the direct effect of endotoxins, or directly from fetal stress due to inflammation of fetal tissues and organs(Samartino and Enright, 1993).

Erythritol, a 4-carbon alcohol, which is a constituent of normal cattle fetal fluids was thought to be responsible for the preferential growth of *Brucella* within placental cotyledon. It was suggested that erythritol enhanced the growth of *Brucella* by providing a unique source of carbon for bacterial metabolism. Other simple sugars such as, glucose, mannose, galactose, fructose and N-acetyl glycosamides failed to stimulate *Brucella* growth(Petersen *et al.*, 2013). Endotoxins of *Brucella* organisms may induce the production of stress hormone called cortisol that leads to decreased progesterone production and an increase in the estrogen production. Decreases in progesterone levels and increase in estrogen levels are known to induce abortions and premature parturitions(A Robinson, 2003).

The outcome of *Brucella* infection depends on the animal species infected, age, immune status of the host, pregnancy status, and the virulence and the number of invading organisms. When the bacteria prevail over the host's defenses of susceptible pregnant animal; bacteremia often leads to the invasion of the uterus. Generally, Localization of *Brucella* bacteria within the female and male reproductive tracts accounts for the most common clinical signs of infection: abortion and male infertility(Poester *et al.*, 2013).

2.5.2. *Clinical sign*

The incubation period of brucellosis varies between 14 and 120 days(Tolosa.T, 2004). The main clinical manifestations in ruminants are abortion and stillbirths, which usually occur in the last third of the pregnancy following infection and usually occur only once in the animal's lifetime(Aparicio., 2013). In camels, the clinical picture of brucellosis can vary from asymptomatic to abortion(Musa *et al.*, 2008). According to various researchers, the clinical signs of brucellosis in breeding camels are the same as those in bovines and small ruminants, although infection in breeding camel causes fewer abortions than it does in cattle and small ruminants(Fowler *et al.*, 2010). Abortion in camel due to brucellosis usually occurs only once. Dams can develop ovario-bursal adhesions, hydro bursitis, and granulomatous endometritis. Placental retention, infertility, and delayed sexual maturity have also been reported(Rafieipour and Ziaei., 2011).

However, placental retention is rare in camel due to the difference in the placental attachment as they possess a diffuse like placenta (Fowler *et al.*, 2010). Brucellosis also causes fetal death and mummification and reduced milk yield. Males may suffer from orchitis, infection of the accessory sex glands, arthritis accompanied by acute lameness (Sprague *et al.*, 2012). Although mammary gland infection may not result in visible clinical symptoms or gross lesions, *Brucella spp* frequently localize in the mammary gland and cause mastitis (Olsen and Tatum, 2010).

In cattle's, gross lesions are not pathognomonic but can include variable necrosis of cotyledons across the placenta and thickening of inter-cotyledonary areas (Megid *et al.*, 2010). In addition to the above stated clinical manifestation, carpal hygromas are a common clinical sign of chronic brucellosis in cattle and can result in joint pain, inflammation, and a reduction in mobility that can be particularly disabling for them. This has the potential to severely limit their usefulness as a means of transportation or draught power and can compromise the income of farmers or community members who depend on them for these purposes (Franc *et al.*, 2018).



Figure 3: Characteristic hygromas in a cow.

Source: Own photo., (2020)

2.6. Diagnosis

Establishment of adequate control programs against brucellosis in a population depends on the presumptive diagnosis of the infection. Brucellosis may be suspected based on clinical signs such as abortion, but confirmation can be made through serological tests. Since 1897, a considerable number of serological tests have been developed. A number of these tests were modified in various ways to increase performance (Nielsen, 2011). Serological tests offer best alternatives to culture and isolation method of diagnosis since the tests are easy to perform, less risky and provide result within a short period. On the other hand, brucellosis can be diagnosed definitively by isolation and identification of the causative organism. This was first reported by Bruce and co-workers in 1887 when they isolated *M. melitensis* from military personnel in Malta (Nielsen, 2011).

2.6.1. Conventional methods (Bacteriological diagnosis)

This refers to isolation and identification of *Brucella* from clinical samples which is always required for the biotyping of strains. The diagnosis of brucellosis by culture and isolation of organisms from clinical samples is the gold standard method. But this method is laborious, time consuming, and risky, whereas the outcome of the test depends on the competence of the laboratory personnel. Valid samples for the isolation of *brucella* bacteria include aborted fetuses (stomach, spleen, and lung), fetal membranes, vaginal secretions, colostrum, milk, sperm, and fluid collected from arthritis or hygroma (OIE, 2018).

At slaughter, in order to confirm suspected cases of acute or chronic brucellosis, the preferred tissues are the genital and oropharyngeal lymph nodes, the spleen, and the mammary gland and associated lymph nodes (Godfroid *et al.*, 2010). For isolation of *brucella*, the recommended medium is Farrell's medium, which contains six antibiotics. But other selective *brucella* media are also in use for the growth of this pathogen from fresh milk and other tissue samples (Radwan *et al.*, 1995). Some *Brucella* species, like *B. abortus* wild type (biovars 1-4), need CO₂ for growth, while others, like *B. abortus* wild type (biovars 5, 6, 9), *B. abortus* S19 vaccine strain, *B. melitensis*, and *B. suis*, do not (Alton *et al.*, 1988).

For liquid samples (milk or blood), sensitivity is increased by the use of a biphasic medium like the Castaneda medium, originally described for use with human blood cultures (Padilla Poester *et al.*, 2010). Growth may appear after 2-3 days, but cultures are usually considered negative after 2-3 weeks of incubation (Alton *et al.*, 1988).

Table 4: *Brucella spp* isolated from cattle in different country.

Country	Species isolated	Sample examined	Test employed	Reference
South Africa	<i>B. melitensis</i> biovar 3	Lymph nodes	PCR	(Kolo <i>et al.</i> , 2018)
Iran	<i>B. abortus</i> biotypes 2, 3, 9	Milk	Culturing	(Zowghi <i>et al.</i> , 1990)
Tajikistan	<i>B. abortus</i> , <i>B. melitensis</i>	Milk	PCR	(Lindahl-Rajala <i>et al.</i> , 2017)
Bangladesh	<i>B. abortus</i> biovar 3	Milk, fetal tissue, Vaginal swab	Culture PCR	(Islam <i>et al.</i> , 2019)
Tanzania	<i>B. abortus</i> biovar 3	Milk Aborted fetus	PCR & MLVA	(C. Mathew <i>et al.</i> , 2015)
Syria	<i>B. melitensis</i>	Milk	PCR	(Al-Mariri, 2015)
Mexico	<i>B. melitensis</i> , <i>B. abortus</i> & <i>B. suis</i>	Manure	PCR	(Morales-Estrada <i>et al.</i> , 2016)

Note: PCR (Polymerase chain reaction), MLVA (Multiple locus variable number tandem repeat analysis).

Table 5: *Brucella spp* isolated from camel in different country

Country	Species isolated	Sample examined	Test employed	Reference
Iran	<i>B.melitensis</i> biovar 2	Milk, placenta Vaginal swab	Culture method	(Zowghi <i>et al.</i> , 2008)
Iran	<i>B.abortus</i> <i>B.melitensis</i>	Blood Lymph node	PCR	(Khamesipour <i>et al.</i> , 2015)
Kuwait	<i>B.abortus</i> biovar 1	Aborted fetus	PCR	(Sultan and Abdalla, 1989)
Saud Arabia	<i>B.abortus</i>	Serum	PCR	(Alshaikh <i>et al.</i> , 2007)
Sudan	<i>B.melitensis</i> ,biovar 3 <i>B.abortus</i> biovar 6	Lymph node	PCR	(Musa <i>et al.</i> , 2008)
Oman	<i>B.melitensis</i>	Aborted fetus Vaginal swab Milk	PCR	(Foster <i>et al.</i> , 2018)
Egypt	<i>B.melitensis</i> biovar 3	Milk	PCR	(Ibrahim1 <i>et al.</i> , 2016)
Iran	<i>B.abortus</i>	Milk	PCR	(Alamian and Dadar, 2019)

Notes: PCR (Polymerase chain reaction), RBPT (Rose bengal plate test), CFT (compliment fixation test)

2.6.2. Serological diagnosis

The majority of studies on animals' brucellosis were conducted by using serological methods for diagnosis in under developed African countries particularly in Ethiopia. To diagnosis brucellosis in camel, none of the serological tests are validated for use as acknowledged by OIE. Similarly, none of the tests have been validated for the diagnosis of human brucellosis according to (Yohannes *et al.*, 2012). However, it was found that a combination of different serological tests can increase diagnostic efficacy in animals, although none of the serological tests can differentiate between a *B. abortus* or *B. melitensis* or *B. suis* infection. On the other hand, false-positive or unspecific reactions with various other bacterial species such as *Yersinia enterocolitica* serotype O: 9 can occur(Chisi *et al.*, 2017).

A) Rose Bengal Plate Test (RBPT)

Among many types of serological test employed for diagnosis of brucellosis in camel and other domestic animals, RBPT is a widely used screening test for regulatory control and export requirements. Rose Bengal Plate Test (RBPT) is one of a group of tests known as the buffered Brucella antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH (Hotam Singh Chaudhary, 2011).

RBPT is a very sensitive test and is suitable for screening herds for brucellosis, but it can give false positive results due to vaccination with *B. abortus* strain 19 vaccine or cross reactions with other bacteria (Omer *et al.*, 2010). Therefore false negative responses are reported to occur less frequently than false positive responses (Omer *et al.*, 2010). It was reported by Chachra *et al.*, (2009) that, among the commonly used conventional sero diagnostic tests for brucellosis, RBPT and STAT may not be absolutely reliable. RBPT detected antibody in the sera of 50% of the animals suspected for brucellosis whereas, STAT could detect only 5.55% cases according to (Chachra *et al.*, 2009) report.

B) Complement Fixation Test (CFT)

The Complement Fixation Test (CFT) allows the detection of anti-Brucella antibodies that are able to activate complement. Many authors regarded the CFT as being the most sensitive and specific test for brucellosis diagnosis. Because CFT antibodies remain in the serum for longer period of time than SAT antibodies (Njeru *et al.*, 2016). On the contrary, some authors disclosed that this test is not highly sensitive but shows an excellent specificity. In the recent year, CFT is progressively being replaced by ELISAs since it is difficult to be standardized. The drawbacks of CFT is the false negative results with the IgG2 type antibodies and the fact that it is technically challenging to perform as a large number of reagents, controls and reagent titrations are required (Sanogo *et al.*, 2013). This test requires good laboratory facilities and trained staff. Prozone formations, cross reactions anti-complementary activities are also problems encountered with CFT (OIE, 2004)

C) Enzyme Linked Immuno sorbent Assay (ELISA)

ELISAs are divided into two categories, the indirect ELISA (iELISAs) and the competitive ELISA (cELISAs). Most iELISAs use purified smooth LPS as antigen and detect mainly IgGs or IgG subclasses. Their main quality is their high sensitivity but iELISA is more vulnerable to non-specific reactions, notably those due to YO9 infection (Godfroid *et al.*, 2010). ELISA was first developed for the diagnosis of human brucellosis. The ELISA tests offer an excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form. A comparison with the SAT, ELISA yields higher sensitivity and specificity. ELISA is also reported to be the most sensitive test for the diagnosis of neuro brucellosis (Miguel *et al.*, 2006). The omp28 protein is now being used in an indirect plate ELISA system and has been evaluated with good sensitivity and specificity on large number of clinical samples (Hotam Singh Chaudhary, 2011). Generally, this primary binding assay (iELISA) is recommended by the OIE as a suitable screening test (Chisi *et al.*, 2017).

D) lateral flow immunochromatography Assay (LFA)

The lateral flow immunochromatography assay (LFA) is a rapid diagnostic test originally developed for the detection of IgM and IgG specific for Brucella S-LPS in human sera (Gusi *et al.*, 2019). LFAs have also been developed for testing other livestock species for brucellosis and its use could be equally attractive (Abdoel *et al.*, 2008). The device consists of a porous nitrocellulose detection strip flanked at one end by a reagent pad and at the other end by an absorption pad. A sample application pad flanks the reagent pad in turn. The composite strip is contained in a plastic assay device with a round sample well positioned above the sample application pad and a test result window positioned above the detection zone of the strip. The detection zone contains two distinct lines, a test line and a control line (Barend *et al.*, 2009). LFA are simple tests with a good performance that do not need special laboratory equipment and only require obtaining serum after clotting (Gusi *et al.*, 2019). It is standardized and evaluated assay for brucellosis with high diagnostic sensitivity, specificity and accuracy for diagnosis of brucellosis in bovines, small ruminants and swine (Shome *et al.*, 2018). Generally; LFA is a useful, rapid, and easy-to-perform tool for the diagnosis of brucellosis (Shome *et al.*, 2018).

2.6.3. Molecular Methods

A) Polymerase Chain reaction (PCR)

The isolation of *Brucella* organisms is still the preferred method of diagnosis. But PCR method allows typing of the isolated strains. PCR based assays have been developed for brucellosis diagnosis and are based on the detection of specific gene sequences of the pathogens. There are numerous reports of diagnostic PCRs in the literature targeting various markers. Two of the most frequently used targets are a 31-kDa immunogenic protein (Bscp31) and the insertion sequence IS711(Whitman, 2015). One of the first PCR assays to differentiate among *Brucella spp* was called AMOS- PCR, developed by Bricker and Halling in 1994. This PCR uses a single reverse primer, targeting the *Brucella* specific insertion elements such as IS711 (Ewalt and Bricker, 2000). However, the most widely applied PCR techniques is the conventional PCR-based Bruce-ladder that can differentiate the major *Brucella* species and vaccine strains(López-Goñi *et al.*, 2011). Even though PCRs can discriminate between *Brucella* species and between wild and vaccine strains, but it does not discriminate between *Brucella* biovars. In recent time new PCR techniques are being implemented for both identification and phenotypic bio-typing(Ron-Román *et al.*, 2019).

B) Multiple Locus Variable Number tandem repeat Analysis (MLVA)

Multiple-Locus Variable number tandem repeat Analysis (MLVA) is a method used to perform molecular typing of particular microorganisms which is developed by Le Fleche and co-workers(Le Flèche *et al.*, 2006). It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in many different loci in the genome of a variety of organisms and is the current gold-standard of *Brucella* typing(Georgi *et al.*, 2017). This method has been used to type various species and strains of *Brucella* with fine scale resolution of closely related isolates (Gyuranecz *et al.*, 2016). Because of its rapidity, highly discriminatory power and reproducibility, it has been suggested that MLVA assay can be useful in epidemiological trace-back analysis of *Brucella* infections with the potential to advance surveillance and control of brucellosis(Al Dahouk *et al.*, 2007).

2.7. Public health importance of brucellosis

2.7.1. Significance and source of infection

Brucellosis in humans is known as "undulant fever" or "Mediterranean fever", "Malta fever" or "Bangs disease"(Zhang *et al.*, 2014). It is a systemic infection that can involve any organ or organ system of the body. Since many cases of brucellosis go unrecognized, the true incidence of the disease is unknown and its zoonotic risk still represents an important public health threat in endemic regions without brucellosis eradication programs(Franc *et al.*, 2018). In human, the disease is common in rural and pastoral areas, because farmers or pastoralists live in close contact with their animals and often consume fresh unpasteurized dairy products. In addition, pastoralist handle aborted fetus with bare hand which is the main predisposing factor of the disease in the area(Zewdie, 2018). Food producing animals such as cattle, sheep, goats, pigs and camel are also the main sources of brucellosis to human being(Galińska and Zagórski, 2013). The type of *Brucella* to which an individual exposed is a significant determinant factor of the risk of disease and its severity in humans. This will be influenced by the species of host animal acting as source of infection(Corbel, 2006).

2.7.2. Interpersonal and occupational transmission

Human to human transmission is extremely rare. However, it can happen trans placentally, via breastfeeding, bone marrow transfer, organ transplantation, blood transfusions and rarely through sexual intercourse (Tuon *et al.*, 2017). Even though, *B. abortus*, *B. suis* and *B. canis* are considered as potential causative agents of brucellosis in human, *B. melitensis* is the most virulent *Brucella* with a few organisms (10 to 100) being sufficient to cause a debilitating chronic infection(Xavier *et al.*, 2014). In human; occupation is the major predisposing factors for brucellosis occurrence. For instance; dairy farmers who milk with bare hands have a greater chance to get infected by *Brucella* from carrier animals as do farmers or slaughterhouse workers who have skin lesions which provide an entry point for the bacteria(Tumwine *et al.*, 2015). In addition, brucellosis vaccines such as Rev-1 and RB51 are live dried vaccines. Thus, accidental self-injection during their preparation or administration could also be a risk factor for human infection(Woodward, 2009).

2.7.3. Manifestation of brucellosis in human

Brucellosis may present with acute or insidious onset, with continued, intermittent or irregular fever of variable duration, profuse sweating, fatigue, anorexia, weight loss, headache, arthralgia and generalized aching. Abscess formation is a rare complication(Seleem *et al.*, 2010). Brucella endocarditis and neurobrucellosis cause most deaths(Pendela *et al.*, 2017). Sometimes, the manifestations of brucellosis are more pronounced in a specific organ system. The most common local manifestations are: spondylitis, peripheral arthritis (especially of the hip, knee and shoulder) and epididymo-orchitis(Colmenero *et al.*, 1996). Arthritis and joint pain are common and usually migratory in character, affecting mostly the large joints, with unilateral joint involvement being more common among the younger age group(Memish and Balkhy, 2004). In General; human Brucellosis can result in osteoarticular, gastrointestinal, respiratory tract, genitourinary, cardiovascular, neurological, cutaneous and ophthalmic complications(Corbel, 2006).

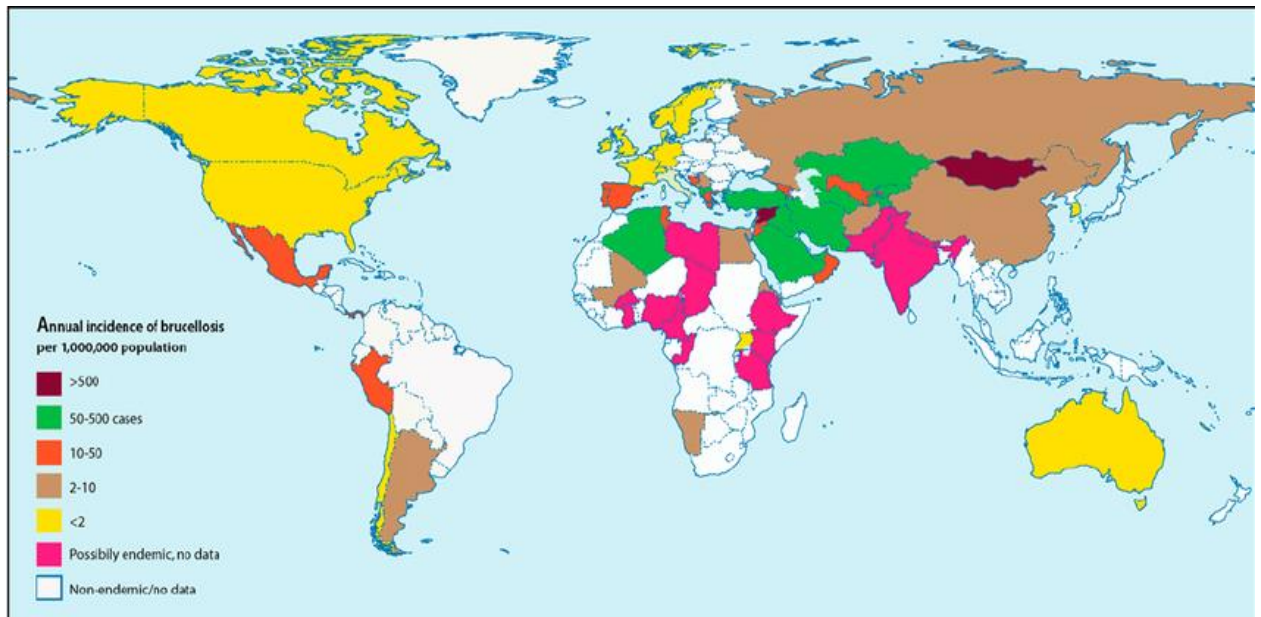


Figure 4: Global incidence of human brucellosis

Source:(Ariza *et al.*, 2007)

In human brucellosis essentially acquired by the oral, respiratory, or conjunctival routes, but ingestion of raw contaminated milk constitutes the main risk to the general public where the disease is endemic. Though camel milk ingestion is a known mechanism for brucellosis acquisition, only a few reports of sporadic cases have been published in the medical literature(Shimol *et al.*, 2012). In Nigeria pastoralist believes that camel milk and urine, when consumed, serve as cure for various diseases including HIV/AIDS, epilepsy and various cancers which can also transmit *Brucella* bacteria to the pastoralist (Salisu *et al.*, 2018). Cheese made from camel milk plays an important role of transmitting *Brucella* bacteria from infected camels (Salisu *et al.*, 2017). There is also an occupational risk to veterinarians, abattoir workers and farmers who handle infected animal carcasses and aborted fetuses or placentas. Brucellosis is also one of the most easily acquired laboratory infections, and all laboratory manipulations with live cultures or potentially infected contaminated material must be performed at an appropriate biosafety and containment level determined by bio risk analysis(OIE, 2018).

2.7.4. Public health importance of brucellosis in Ethiopia

As it was stated above, pastoral community of Ethiopia, mainly depends on camel and other domestic animals milk and milk product to fulfill their dietary requirement which is the well-known transmission route of brucellosis from animals to human(Cossins and Upton, 1987). On the other hand, traditional type of food animal slaughtering in non-hygienic methods are common practices which definitely downgrade the hygiene, safeness and wholesomeness of food of animal origin. Consumption of such contaminated food which may contain *Brucella* bacteria has the potential to cause an adverse health effect(Desta, 2016).

Majority of Somali, Oromia, and Afar regional state pastoralists do not use any protective materials during handling parturient animals, removing placenta and/or other aborted materials since most of the people had poor knowledge about brucellosis(Desta, 2016). So, these practices could potentially facilitate the transmission of zoonotic *Brucella* pathogens from domestic animals' to humans (Bekele *et al.*, 2013). Generally, human brucellosis is increasing in Ethiopia like many other developing countries due to various sanitary, socioeconomic, and political factors (Pappas *et al.*, 2006).

Thus, collaborative work of different stakeholders to prevent and control the disease as well as to enhance public awareness level of livestock keepers is required (Catley *et al.*, 2005).

Table 6: Sero prevalence of human brucellosis in pastoral and abattoir workers in Ethiopia

District	N₀: examined	Sample taken	Test employed	prevalence	Reference
Fafan zone	211	serum	CFT	0.4%	(Lakew <i>et al.</i> , 2019)
Afar	200	serum	RBPT	16%	(Zewolda and Wereta, 2012)
			CFT	15%	
	630	Serum	RBPT	12.7%	(Zerfu <i>et al.</i> , 2018)
Bishoftu	80	Serum	CFT	35%	(Tsegay <i>et al.</i> , 2017)
	149	serum	RBPT	4.7%	
Modjo			CFT	1.3%	
Addis Ababa	360	serum	RBPT	-	(Kassahun <i>et al.</i> , 2006)
			2-MET	4.8%	

Notes: N₀. examined (Number of humans examined).

2.8. Treatment of Brucellosis

2.8.1. Treatment of brucellosis in Animals

As a general rule, treatment of infected livestock is not attempted because of the high treatment failure rate, cost, and potential problems related to maintaining infected animals in the face of ongoing eradication programs (Yousefi-Nooraie *et al.*, 2012). In developed countries, treatment of infected animal is not a common practice. However, the infected animals are isolated, culled or slaughtered to prevent the spreading of infection to another herd. Even though the complex nature of brucellosis makes it difficult to treat, long term treatment with an antibiotic is thought to be beneficial to care for economically valuable breeding male animal and must be instituted before irreparable damage to the epididymis has occurred (Alemneh and Akebergn, 2018).

2.8.2. Treatment of brucellosis in Human

Humans are treated with antibiotics (doxycycline with rifampicin) even though relapses are possible. Several conventional antibiotics including tetracyclines, trimethoprim, sulfamethoxazole, amino-glycosides, rifampicin, quinolones, chloramphenicol, doxycycline, and streptomycin are commonly used in clinics (Saltoglu *et al.*, 2002). The World Health Organization recommends that acute brucellosis cases should be treated with oral doxycycline and rifampicin (600 mg for six weeks) (Ersoy *et al.*, 2005). However, rifampicin monotherapy is in common practice for treating brucellosis in pregnant women, and combined therapy of sulphamethoxazole and trimethoprim is recommended for children (Karabay *et al.*, 2004).

2.9. Prevention and control of livestock brucellosis

Although brucellosis has been controlled in most industrialized nations, the disease has become a neglected zoonosis in some tropical or developing countries due to lack of sustainability in the disease prevention and control programs (Ekere *et al.*, 2018). According to Zhang *et al.*, (2018) report, brucellosis control or eradication programs, the implementation of the programs, and the control measures in different countries vary greatly depending on their own national conditions. Even with the high economic burden of the disease in many low-income countries, the disease does not attract the appropriate attention of national health systems (Godfroid *et al.*, 2013).

In the developed world, the control of animal brucellosis has been approached with a combination of procedures such as: vaccination, test and slaughter programs whereas human brucellosis through milk pasteurization and hygienic measures coupled with effective disease surveillance and animal movement control (Godfroid *et al.*, 2011). Control of camel brucellosis should be tailored to suit conditions in the particular countries where animals are kept. Most of these countries are poor and livestock are kept mainly by nomadic tribes. So control of livestock brucellosis can be achieved through extending veterinary services (Abbas and Agab, 2002).

It was suggested that, the preferred control strategy of camel and cattle brucellosis should be based on whole herd vaccination using S19 or Rev 1 vaccinal strains preceded by blood testing using the SAT or card test on the field. Seropositive animals should be identified by branding or special ear-mark and subjected to retesting. This marking will restrict the sale of seropositive animals.

2.9.1. Vaccination

Animal brucellosis control strategies differ in the developed and developing world. In developed world, most emphasis is given to eradication and risk analysis to avoid the re-introduction of *Brucella* while information related to the prevalence of brucellosis is still scarce and control programs are rarely implemented in developing world (Franc *et al.*, 2018). However, vaccination is the cornerstone of control programs to prevent brucellosis in livestock in both developed and developing world. So, serious efforts of vaccination have been made to prevent the infection through the use of vaccines(Wernery, 2016). Before vaccination is started in livestock, thorough investigations are paramount important, in order to find out whether the animals are naturally infected by *B. abortus* or *B. melitensis* and this can only be determined by culture or PCR. An eradication campaign in livestock may also be based on vaccination (Wernery, 2016).

According to Radwan *et al.*, (1995), vaccination of camel with Rev 1 found to be effective, safe, successful and economically acceptable methods of controlling brucellosis in Saud Arabia. It is obvious that, brucellosis in cattle is almost exclusively caused by *B. abortus* (Muradrasoli *et al.*, 2015), but *B. melitensis* and *B. suis* have been implicated in some herds making the vaccination of cattle using vaccines targeting only *B. abortus* less effective in preventing brucellosis in cattle and transmission to humans(Moriyón *et al.*, 2004). Generally, the main approach in a long-term control strategy of brucellosis is to vaccinate only female replacement animals, specially, camel less than 1-year-old (maturity in OWCs begins with 4 years). Camel calves should be vaccinated at 4-8 months of age, using a full adult dose of vaccine(Dorneles *et al.*, 2015). After several years, this strategy will establish an immunized herd and will not induce abortions. It will also protect these herds from brucellosis threat by surrounding positive livestock(Dorneles *et al.*, 2015)

In countries where eradication in animals through vaccination and/or elimination of infected animals is not feasible, prevention of human infection is primarily based on raising awareness, food-safety measures, occupational hygiene and laboratory safety. In most countries, brucellosis is a notifiable disease(Nematollahi *et al.*, 2017).

2.9.2. Test and Slaughter

Test and slaughtering of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (Luelseged, 2019) which is feasible only in developed world (McDermott and Arimi, 2002). The decision about slaughtering of test positive animals is made after regulatory, economic and prevalence factors are considered. In developing countries, the isolation of test positive animals is essential, especially during and after parturition since immediate slaughtering of test-positive animals is expensive and requires animal owner cooperation(Luelseged, 2019). This indicates that, livestock keepers in developing countries cannot afford the traditional test and slaughter approach especially when expensive animals with high genetic potential are involved(Radwan *et al.*, 1995). Furthermore, the application of test and slaughter policies work well only under reliable diagnostic tests to avoid unnecessary decision due to false positivity(Alp *et al.*, 2006).

2.9.3. Hygienic prophylaxis

Application of hygiene measures to the control of brucellosis become successful through the reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control. Factors such as the methods of animal husbandry (e.g., commingling of herds or flocks), patterns of commerce, type of facilities, and degree of dedication of the owners of animals, will also determine success. However, owners have poor understanding about the transmission route of brucellosis in livestock so that, separation of parturient animals, can be difficult or even impossible to implement(Glynn and Lynn, 2008) which is a conspicuous existing gap.

3. MATERIALS AND METHODS

3.1. Description of the study area

Afar National Regional state is located in the eastern part of the country between 39° 34' and 42° 28' E longitude and 8° 49' and 14° 30'N latitude. The total geographical area of the region is about 270,000 km² and more than 90% of the residents of the region are pastoralist. The remaining 10% are agro-pastoralists. The region is bordered by Eritrea on North-East and with Djibouti on the East , with Tigray Regional state on the North-West, with Amhara Region on the South-West and with Oromia Region on the South and with Somali Region on the South East of Ethiopia (Tekle *et al.*, 2019).

The region is characterized by arid and semi-arid climate with low and inconsistent rainfall. The altitude of the region ranges from 120m below sea level to 1500m above sea level. Temperatures vary from 20°C in higher elevations to 48°C in lower elevations. Rainfall is bi-modal throughout the region with a mean annual rainfall below 500mm in the semi-arid western escarpments and decreasing to 150mm in the arid zones to the East. Based on the 2017 projections by the Central Statistical Agency of Ethiopia (CSA), the region has a total population of 1.8 million and administratively, divided into five zones, which are further subdivided into 34 woredas (administrative districts) and 358 pastoral associations (Tekle *et al.*, 2019 ; CSA, 2013).

This study was conducted in Amibara district of Gabirasu zone (Zone 3) which is located in the Middle Awash Valley. Amibara district is about 250km to the North East of Addis Ababa and has 19 kebeles (the least administrative units) with total population of ~63,378, of which 35,374 were men and 28,004 women. The altitude of Amibara district is 740m above sea level. Fourteen years climatic data on monthly basis showed that the average maximum and minimum temperature of the area is 34°C and 19°C, respectively, and the annual total rainfall in the area is about 571 mm(Chekol and Mnalku, 2012).The livestock populations of the Amibara district are composed of 103, 959 cattle, 122, 526 goats, 48,043 sheep, 3,888 donkeys and 39,995 camels (CSA, 2007).

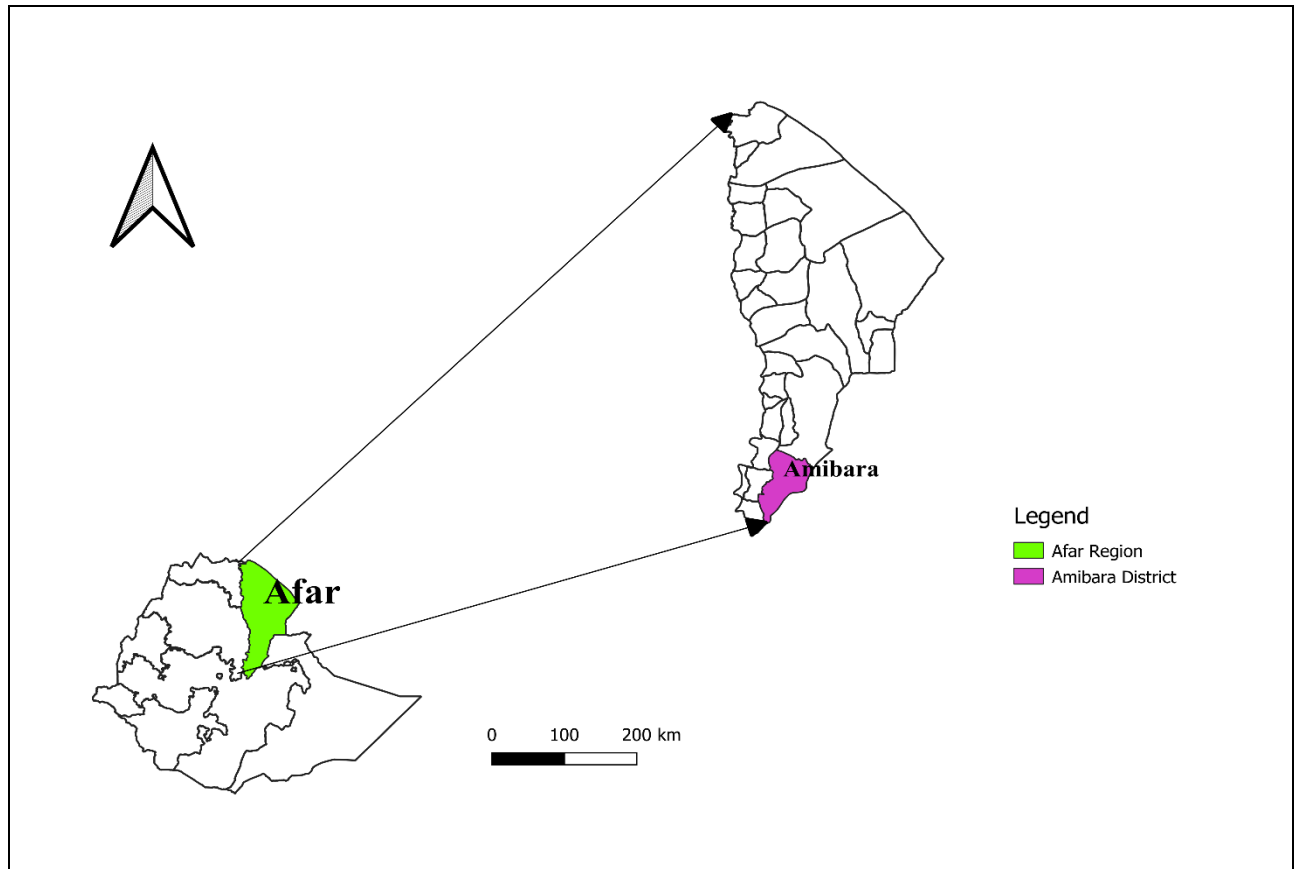


Figure 5: Map showing the Afar Regional State and proposed study district

Source: Diva GIS

3.2. Study Population

3.2.1. Animal study population

The target study populations were cattle and camel herds in the hands of pastoralists including those with history of abortion in Amibara district of Afar region, Ethiopia. Cattle and camels under study comprised only indigenous local breed with no history of vaccination. Both cattle and camels older than six months of age were included into the study as the disease wasn't common in the animals less than 6 months of age due to maternal antibody. Cattles were classified into three age groups (<2 years, 2-5years and >5 years) as young ,adult and old respectively based on(Ibrahim *et al.*, 2011) whereas camels classified into (≤ 4 years, 4-10 years and >10 years) as young, Adult and old age group according to (Gizaw *et al.*, 2017).

Information related to environmental and study animals such as kebele, sex, body condition, reproductive status, parity number and placental retention were collected and recorded at the time of sampling. The body condition of cattle and camels was classified into three as good, medium and poor based on the description of Lowman *et al.*, (1976). Additionally, the owner name and their family members were recorded to avoid double sampling from each herd considering the mobile lifestyle of the society.

3.2.2. Human Population

In human, the target study population was the owners of the sampled camels and cattle in the study area. Maximum of four individuals were recruited into the study from each house hold based on their interest. Patients visiting the local health station and interested individuals having history of contact with infected animals and consumption of raw animal product were also considered as a target population.

3.3. Study design and sample size determination

A cross-sectional study design was used from October 2019 to May 2020 in order to determine seroprevalence of cattle and camel and the owners of sampled animals including interested individuals in Amibara district of Afar Region. Study kebeles were selected by two stage cluster sampling technique considering livestock potential and accessibility. Then kebeles having highest livestock population were selected by simple random sampling technique from appropriate cluster. To select camel herds in the proposed kebeles, purposive sampling techniques was used based on the willingness of herd owners to cooperate, presence of aborted she camel in the herd and accessibility during the period of study. Then each herd was stratified into subgroup based on age and sex to ensure equal representation of all the subgroup. From each subgroup, individual animals were selected by systematic random sampling technique. To select cattle herds for serological study, villages in the chosen kebeles were selected purposively based on livestock potential and accessibility, preference by pastoralist during seasonal migration including reported case of abortion. From the selected villages, existing households at the time of study were sampled by simple random sampling technique.

After households were selected, the procedure used for sampling individual camel was also used for cattle sampling. Information related to environmental and study animals such as kebele, sex, age, body condition (Lowman *et al.*, 1976), reproductive status, parity number and placental retention were collected and recorded at the time of sampling. Additionally, the owner name and their family members were recorded to avoid double sampling from each herd considering the mobile lifestyle of the society.

3.3.1. Sample size determination of study animals

The sample size for serological study of brucellosis in cattle was estimated based on the previous study report by (Asgedom *et al.*, 2016) in Alage district which is 2.4% where as 4.1% seroprevalence was reported in camels by (Hadush *et al.*, 2013) and (Gizaw *et al.*, 2017) in Afar region.

Therefore, the minimum required sample size was calculated using the formula described by (Thrustfield, 2007), with defined precision of 5 % and level of confidence interval of 95%.

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

Where

n = required sample size,

P_{exp} = expected prevalence, and

d = desired absolute precision

So, based on the above formula and taking the reported seroprevalence of cattle and camel as 2.4% and 4.1% respectively, the minimum required sample size was calculated for both animals' species separately.

$$n(\text{Cattle}) = \frac{(1.96)^2 * 0.024(1 - 0.024)}{0.05^2} = 36$$

$$n(\text{Camel}) = \frac{(1.96)^2 * 0.041(1 - 0.041)}{(0.05)^2} = 61$$

However, in order to increase precision and reduce standard error, the minimum sample size obtained by calculation were increased by four-fold. Therefore, about 181 cattle and 250 camel were considered for this study from selected kebeles of Amibara district.

3.3.2. Sample size determination of human participant

To select human participant, Purposive/convenience sampling technique was employed. All the owners of sampled animals were embraced into the study based on their interest. The sample size of human participant was also determined based on the finding of (Eshetu *et al.*, 2018) who reported 4.8% seroprevalence in Eastern Ethiopia. By taking the above reported seroprevalence of brucellosis in human, the minimum required sample size was calculated by using (Thrustfield, 2007). Hence, a minimum of 70 individuals were required. In order to increase precision and reduce standard error 120 participants were included into the study.

3.4. Blood sample collection

3.4.1. Blood sample collection from animals

Both female camel and cattle with history of abortion were sampled purposively whereas individual animals without the stated reproductive health problem were recruited into the study by systematic random sampling technique. Before commencing draining of the blood sample, each animal was isolated and restrained properly to avoid unexpected personal injury and to minimizing unnecessary stress that might be happen to the animals. After shaving and disinfecting the site of jugular vein, 10 ml of blood sample was collected into sterile plain vacutainer tube from each study animal. The sample was labeled by using code describing herd number, species and kebele and placed in the rack by maintaining protection from exposure to direct sun light and extreme temperature.

Then; the samples were taken to Werer Agricultural Research center (WARC) animal health laboratory within a few hours on daily basis (“LabCorp,” 2019) and kept in a slanted position overnight at room temperature to separate the serum and the clotted red blood cells according to (OIE, 2018). On the next day, the clotted blood samples were centrifuged at 1500 rpm for 5 minutes to obtain clear serum. Finally, the serum was gently decanted into sterile cryovials (1.8ml), labeled and stored at -20⁰c in WARC animal health research laboratory until it gets transported to National Veterinary Institute (NVI), serology department, Bishoftu, Ethiopia.

3.4.2. Collection of blood sample from human participants

In human, about 5-7 ml of peripheral blood sample was collected from each respondent preceded by verbal agreement and getting permission from each. Nurses working in Melka Werer Health station collected blood samples from the respondents. After blood sample collection, the sera were separated from the blood after allowing to stay in slanted position at room temperature for 24hrs and centrifuging at 1500 rpm for 5 minutes. Finally, the serum from each sample was decanted into the cryovials (1.8 ml) labeled, packed and stored in WARC Animal health research laboratory at -20⁰C.

3.5. Questionnaire survey

In the present study, the owners of the sampled camels, cattle and interested individuals were interviewed using structured questionnaire (Annex 7). The questionnaire format was focused on the knowledge and awareness related with the transmission of brucellosis from livestock to human which covers demographic data variables (age, sex, educational level, occupation, marital status), consumption habit (raw milk, meat and other product), contact with aborted/new born calf. Information’s related to animal keeping, sheltering including their knowledge of major animal diseases which can be transmitted to humans were also collected.

3.6. Laboratory Diagnosis

3.6.1 Serological tests

In this study, cattle and camel sera were diagnosed by RBPT as screening and CFT as confirmatory test at National Veterinary Institute (NVI) serology laboratory, Bishoftu, Ethiopia. But human sera were screened by RBPT at Werer Agricultural Researcher Center (WARC) and positive to RBPT were further confirmed by CFT.

A) Rose Bengal Plate Test (RBPT)

All serum samples of cattle and camel were screened using RBPT at NVI according to the procedures described by the World Organization for Animal Health (OIE, 2018). In the laboratory, serum samples and antigen were kept at room temperature for 30 minutes and then, screened for anti-Brucella antibodies using commercial kits of the standard Rose Bengal Plate Test (RBPT). *B. abortus* antigen (Lillidale Diagnostics, Holt wimborne, Dorset, BH21 7DG, United Kingdom) and their positive and negative control sera were used to detect the Brucella antibodies following the manufactures' instructions. The detailed procedures for the RBPT was, 30µl of serum sample and 30µl of antigen was mixed on a test plate and rocked for 4 minutes. After four minutes of rocking, visible agglutination was considered as positive.

Agglutinations were recorded as 0, +, ++ and +++, according to the degree of agglutination (Nielsen, 2002). A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination, and +++ indicates coarse clumping. The presence of agglutination at any degree was considered as positive reaction while the absence of agglutination was considered as negative. Finally, the results were recorded and stored in Microsoft Excel.

B) Complement Fixation Test (CFT)

Serum samples that reacted positively to RBPT were further tested by CFT for confirmation using standard *B. abortus* antigen S99(New Haw, Addlestone, Surrey, KT15, 3NB-UK). The detailed procedure of CFT was indicated in (Annex 4). Preparation of the reagent is evaluated by titration and was performed according to protocols recommended by World Organization for Animal Health(OIE, 2008). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above was considered as positive and lack of fixation/complete hemolysis was considered as negative result. Only samples that gave signals for both RBPT and CFT were considered positive since no single test is appropriate in all epidemiological situations due to problems of sensitivity and or specificity of the tests as recommended by OIE and other reports(Tumwine *et al.*, 2015).

3.7. Data Analysis

Putative factors believed to be associated with the occurrence of brucellosis, serological results and questionnaires data were recorded in a Microsoft Excel® Spread Sheet. Serological and questionnaire data were entered into Microsoft Excel spreadsheet and analysis was done using R-Software version 4.0.0. Prevalence was calculated by dividing the number of combined RBPT and CFT positive animals /humans by the total number of animals/humans tested. Herd level prevalence was calculated by dividing the number of herds with at least one positive animal by combined RBPT and CFT to the total number of herds tested.

Fisher's exact test was used to calculate associations of risk factors with brucellosis seropositivity for camels, cattle and human data. In the current study, the number of outcomes of interest was less than 10% of the total sample size of cattle, camel and human. Under this situation, ordinary logistic regression model was not suitable and firth's bias reduced logistic regression model was used instead to measure the association of potential risk factors with brucellosis seropositivity (Puhr *et al.*, 2017).

Odds ratio (OR) was used to measure the degree of association between risk factors such as herd size, age, sex, body condition, parity number, abortion history and RFM with animal level seroprevalence. P-value less than 0.05 was considered statistically significant in all analysis.

3.8. Ethical consideration

The protocol for field studies and collection of animal materials was approved by animal research ethical review committee of the College of Veterinary Medicine and Agriculture (CVMA) with certificate reference number of VM/ERC/03/01/12/2020. For questionnaire survey, pastoralists were informed of the aim of the study and their verbal consent was sought before commencement of data collection (Annex 6). For collection of blood sample from the human participants, the work had earned recognition and written consent obtained from Afar Regional Health Bureau by ref number QAPB011/3934.

4. RESULT

4.1. Brucellosis seropositivity in cattle and camels and associated risk factors

In the present study, the seroprevalence of brucellosis at animal level was computed by using combined RBPT and CFT. Among 431 serum samples from both clinically aborted and non-aborted animals (cattle and camels) tested, 38 were found positive for brucellosis by RBPT and 12 were confirmed by CFT. Thus, the overall seroprevalence of camel brucellosis in the current study was 7.6% (95% CI:0.049-0.1156) using RBPT and 3.2% (95% CI: 0.0163-0.062) by combined RBPT and CFT. In cattle, it was 10.5% (95% CI:0.0683-0.1582) using RBPT and 2.2% (95% CI:0.0086-0.0553) by combined RBPT and CFT.

Table 7 present the result of animal level brucellosis seropositivity in both cattle and camel including the association of abortion with brucella infection. The result showed that, there is no statistically significant difference of brucellosis seropositivity between camel and cattle ($p > 0.05$). However, a greater number of camels were found brucellosis seropositive by combined RBPT and CFT when compared to cattle. On the other hand, abortion history was considered and found significantly associated with brucellosis seropositivity ($P=0.00^*$) in both camel and cattle which means that, cattle and camel with history of abortion were more at risk for brucellosis than non-aborted animals. It was also indicated that, female animals having a history of abortion were 38.9 times more at risk of being tested positive for brucellosis than non-aborted animals (OR=38.9, 95%CI=7.95-375.7)

Table 7: The overall seroprevalence and association of abortion with brucellosis in camel and cattle

Variables	Number tested	No of RBPT positive (%)	No of CFT Positive (%)	P-value	OR (95% CI)
Species				0.537	0.7(0.203-2.306)
Camel	250	19(7.6)	8(3.2)		
Cattle	181	19(10.5)	4(2.2)		
Abortion History**				0.00*	38.9(7.95-375.7)
Yes	57	24(42.1)	10(19.23)		
No	352	12(3.4)	1(0.284)		

** Only female animals were considered, * significant,

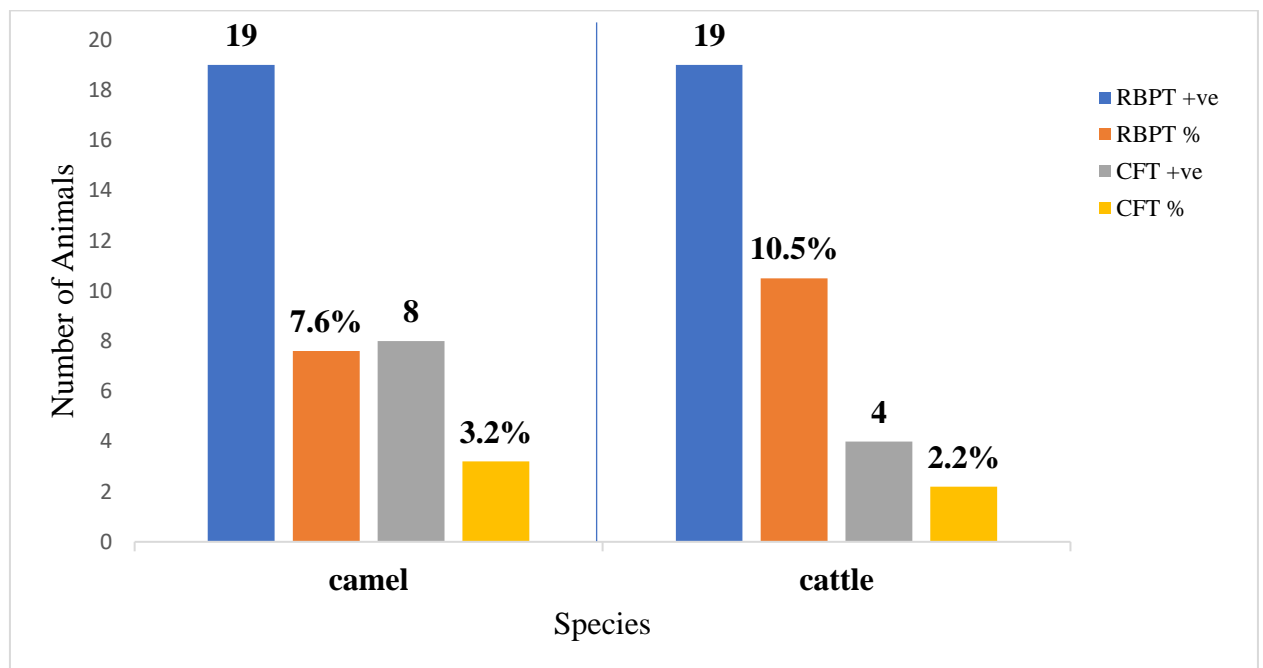


Figure 6: Graphical representation of brucellosis seroprevalence in cattle and camel

Table 8 shows association of risk factors with brucellosis seropositivity in cattle based on fisher exact test. As a result, environmental and different host risk factors were considered and only age of the animals ($\chi^2_{b}=6.77$, $P=0.021$), number of parity ($\chi^2_{b}=9.433$, $P=0.004$), abortion history

($\chi^2=16.02$, $P=0.002$) and placental retention ($\chi^2=19.1$, $P=0.003$) found significantly associated with brucellosis seropositivity in cattle. Even though the sex of animals didn't show significant associated with brucellosis seropositivity, a greater number of female cattle were tested positive for the disease than male.

Table 8: Association of risk factors with brucellosis seropositivity in cattle

Variables	No tested	Seropositive	Prevalence (%)	Fisher exact test value	P-value
Kebeles				3.364 ^b	0.442
Angilale	42	1	2.38		
Badahmo	28	2	7.14		
Bonta	30	0	0		
Halaydgie	50	1	2		
Sidhafage	31	0	0		
Sex				1.948 ^a	0.259
Male	13	1	7.7		
Female	168	3	1.78		
Age				6.77 ^b	0.021*
Young	30	0	0		
Adult	123	1	0.8		
Old	28	3	10.7		
Body condition				1.462 ^b	0.481
Poor	46	0	0		
Medium	107	3	2.8		
Good	28	1	3.57		
Herd size				0.364 ^b	1
<20	44	1	2.27		
20-50	90	2	2.22		
>50	47	1	2.13		
Number of Parity**				9.433 ^b	0.004*
0	25	0	0		
1-5	117	1	0.85		
>5	26	2	7.69		
Abortion History**				16.02 ^b	0.002*
Recent Abortion	21	3	14.3		
No Abortion	147	0	0		
P. retention**				19.1 ^b	0.003
Yes	4	2	50		
No	162	1	0.167		

No test: Total number of animals tested. ^b-Fishers exact test value, ^a- Chi- square value, *Significant;

** Only female animals considered.

4.2. Multivariable analysis of risk factors associated with brucellosis in cattle

By computing Firth's bias reduced logistic regression, only three variables were considered important by the final model in cattle data. As a result, multiparous animals (>5) and animals with history of abortion showed statistically significant association with brucellosis (Table 9). It was also indicated that, adult animals were 16.64 times more at risk of contracting brucellosis when compared with young animals even though it didn't show statistically significant association. In addition, multiparous cattle were 12.2 times and animals with history of abortion 41.4 times more at risk of brucellosis when compared with young animals and those with no history of abortion respectively (Table 9).

Table 9: Multivariable Firth bias reduced logistic regression analysis of factors associated with brucellosis seropositivity in cattle

Variables	Number tested	Seropositive (%)	Adjusted OR	(95%CI)	P-value
<i>Age</i>					
Young	25	0(0)	1	1	-
Adult	117	1(0.85)	16.64	(-0.242,7.849)	0.070
Old	26	3(11.54)	0.12	(-2.816, 7.832)	0.273
<i>Parity number**</i>					
Null	25	(0)	1	1	-
1-5	116	0(0)	0.003	(-11.8, -2.18)	0.383
Greater than 5	27	3(11.11)	12.2	(-7.47,3.17)	0.0012*
<i>Abortion history**</i>					
Yes	21	3(14.28)	41.14	(1.16, 8.69)	0.003*
No	147	0(0)	1	1	-

**Only female cattle were considered. ,1: Reference

Table 10 indicates the association of potential risk factors with brucellosis seropositivity in camel based on fisher exact test. As a result, no statistically significant difference of brucellosis seropositivity was observed between both sexes of camel ($p > 0.05$). However, all of the seropositive camels were female and no male animal was tested positive for the disease. Additionally, analysis of association between brucellosis seropositivity of camel and other potential risk factors was also computed. Consequently; age ($P=0.004$), history of abortion ($P=0.004$), body condition ($P=0.0022$) and number of parity ($P=0.0008$) were found significantly associated with brucellosis seropositivity in camel. However, kebeles, herd size and placental retention were not stand significantly with brucellosis in camels ($P > 0.05$).

Table 10: Association of risk factors with brucellosis seropositivity in camel

Variables	Number tested	Seropositive	Prevalence (%)	χ^2	P-value
<i>Kebeles</i>				5.280 ^b	0.3003
Angilale	75	2	2.67		
Kusura	14	0	0		
Bonta	64	1	1.56		
Halaydgie	73	3	4.1		
Badahmo	11	0	0		
Sidhafage	13	2	15.4		
<i>Sex</i>				0.309 ^a	1
Male	9	0	0		
Female	241	8	3.43		
<i>Age</i>				10.7 ^b	0.004 [*]
Young	34	0	0		
Adult	165	2	1.21		
Old	51	6	11.764		
<i>Body condition</i>				10.26 ^b	0.0022 [*]
Poor	53	6	11.3		
Medium	158	2	1.266		
Good	39	0	0		
<i>Herd size</i>				0.926 ^b	0.6357
<20	42	2	4.76		
20-50	92	2	2.17		
>50	116	4	3.45		
<i>Number of Parity**</i>				11.2 ^b	0.0008 [*]
Null	32	0	0		
Less than or =3	160	2	1.25		
Greater than 3	49	6	12.24		
<i>Abortion History**</i>				29.96 ^a	0.004 [*]
Aborted	37	7	18.92		
No Abortion	213	1	0.47		
<i>Placental retention**</i>				0.134 ^b	1
Yes	4	0	0		
No	229	8	3.5		

^bFishers exact test value, ^a- Chi- square value, ^{*}Significant; ^{**} Only female considered

4.3. Multivariable analysis of risk factors associated with brucellosis in camel

After conducting stepwise model simplification, only two variables (parity number and abortion history) were retained in the final model from univariable analysis. Then, multivariable firth's bias reduced logistic regression analysis was computed to evaluate association of risk factors with brucellosis seropositivity in camels. As a result, only history of abortion remained to independently associated with brucellosis seropositivity in camel ($P < 0.05$). Briefly, camels with history of abortion were 49.6 times more at risk of contracting brucellosis than those with no history of abortion. However other factors were insignificantly associated with brucellosis in camels ($P > 0.05$). Camels with number of parity greater than 3 were 2.75 times more at risk of contracting brucellosis than young camels (Table 11).

Table 11: Multivariable firth bias reduced logistic regression analysis of factors associated with brucellosis Seropositivity in camel

Variables	Number tested	seropositive	Adjusted OR	(95%CI)	P-value
Parity Number					
Null	32	0	1	1	-
Less than or =3	160	2	0.15	(-5.31,3.233)	0.357
Greater than 3	49	6	2.75	(-195, 6.00)	0.511
Abortion History					
Yes	36	7	49.6	(2.148,6.34)	0.0002*
No	205	1		1	-

1: Reference

4.4. Serological results of human brucellosis

4.4.1. Socio-demographic characteristics

A total of 120 human participants were involved into the study. Among these, 90 of them were the owners of sampled camels and cattle. The mean age of respondent was (38.44 ± 1.08) that ranged from 19 to 72 years. The majority 65.8% (n = 79) were in 20-59 age group. About 79.2% of the respondents were male (n = 95). Concerned with educational status of the respondents, 67.5%(n=81) were illiterate whereas only 32.5%(n=39) of them had got compulsory education among which few of them were graduate from higher education. The Majority of the respondent 75%(n=90) were pastoralists followed by governmental employee 18.3%(n=22). The average number of animals owned by each respondent is $(47.08 + 4)$. Only 6.7%(n=8) of them had informal occupation mainly casual jobs like looking after livestock, loading and offloading goods in WARC and running daily based and non-permanent work. In this study 85.8%(n=103) of the respondent were married with 4.13(95% CI=3.77, 4.63) over all average family size whereas 0.9% (n=1) respondent was divorced.

Similarly, the environmental and socio- demographic characteristics of the respondent such as kebele, gender, age, family size, educational status, occupation and marriage were considered as a major risk factor. As a result, only occupation found significantly associated with brucellosis seropositivity in humans(P=0.028) based on fisher's exact test (Table 12). Additionally, age of the respondent showed marginally significant association with brucella infection in humans(P=0.057). Even though no individuals from Angilale, Badahmo and Bonta kebele were tested positive for brucellosis, the highest seropositivity of brucellosis was recorded in Halaydge kebele (9.52%) followed by Sidhafage and Werer respectively (Table 12).

Table 12: Descriptive socio-demographic characteristics and brucellosis sero-prevalence among the respondent.

Variables	No tested	RBPT +ve		CFT +ve		χ^2	P-value
		n	%	n	%		
Kebele						3.52 ^b	0.650
Angilale	14	2	14.28	0	0.0		
Bahadamo	20	0	0.0	0	0.0		
Bonta	18	2	11.11	0	0.0		
Halaydge	21	3	14.28	2	9.52		
Sidhafage	19	1	5.26	1	5.26		
Werer	28	4	14.28	1	3.57		
Gender							0.19
Male	95	8	8.4	2	2.1		
Female	25	4	16	2	8		
Age						5 ^b	0.057
13-19 years	6	1	16.67	1	16.67		
20-59years	79	6	7.6	1	1.26		
Above 60 years	35	5	14.3	2	5.71		
Educational status						3.7 ^b	0.786
Degree	1	0	0.0	0	0.0		
Diploma	3	0	0.0	0	0.0		
High school	10	0	0.0	0	0.0		
Elementary	16	2	12.5	1	6.25		
Read and write	9	1	11.1	0	0.0		
Illiterate	81	9	11.1	3	3.7		
Occupation						6.925 ^b	0.028*
Pastoralist	90	7	7.78	2	2.22		
Government	22	2	9.09	0	0.0		
Others	8	3	37.5	2	25		
Marriage						2.6 ^b	0.484
Married	102	11	10.78	3	2.94		
Divorced	1	0	0.0	0	0.0		
Single	17	1	5.88	1	5.88		
Family size						1.694 ^b	0.375
1-2	30	4	13.33	2	6.67		
3-5	58	6	10.34	1	1.72		
Above 6	32	2	6.25	1	3.12		

^bfisher exact test value, No-tested= Number of tested individuals

4.5. Multivariable analysis of risk factors associated with brucellosis in human

Following computation of univariable firth's bias-reduced logistic regression analysis, all environmental and socio-demographic risk factors were insignificantly associated with brucellosis seropositivity in human. However, participant from Halaydge kebele were 6.964 times and those from Werer kebele were 2.27 times more at risk of contracting brucellosis when compared with participant from Angilale kebele. It was also indicated that, individuals with non-permanent work were 18.8 times more at risk of contracting brucellosis when compared with governmental employee and also showed statistically significant association ($P=0.029^*$, 95% CI: 1.324, 2730.32) with brucella infection (Table 13).

Table 13: Multivariable firth's bias reduced logistic regression analysis of factors associated with brucellosis seropositivity in human

Variables	No of tested	seropositive	Adjusted OR	(95% CI)	P-value
Occupation					
Government	22	0	1	1	-
Others	8	2	18.85	(1.324, 2730.32)	0.029*
Pastoralist	90	2	1.42	(0.11, 197.86)	0.817

No of tested=Number of Individuals tested, OR=Odds Ratio, 1: Reference

4.6. Results of questionnaire survey

Questionnaires were also administered to 120 respondents to gather information on their knowledge, attitude and practice about brucellosis and management of their livestock. Information related to raw milk and uncooked meat consumption, sheltering and herding practice, their knowledge of zoonotic diseases specially brucellosis, handling of dystocia cases and their usage of protective gloves were assessed. As a result, 80% of the respondents have no information of the disease transmission from wild to domestic animals (Table 14). Additionally, 76.47% of the respondent told that, they herd their animals by mixing with different domestic animal species. Moreover, majority of the respondent told that, they keep their animals in national park and practice

inappropriate disposal of the aborted fetus and placental membrane which are the major predisposing factors for the occurrence of brucellosis in cattle and camel (Table 14).

Table 14: Frequencies and percentages for the questionnaire survey of respondent of camel and cattle brucellosis in the study area.

Variables	Response category	frequency	%
knowledge of disease transmission from wild to domestic animals			
	Yes	24	20
	No	96	80
How do you herd animals (N=102)			
	Mixed at gazing point	78	76.47
	Mixed at watering point	7	6.86
	All separately	22	21.57
	No idea	13	12.74
Do you Keep animals in Nation park* (N=90)			
	yes	80	88.89
	No	10	11.11
Frequent occurrence of abortion** (N=102)			
	Yes	68	66.67
	No	34	33.33
Which animals frequently abort** (N=102)			
	Cattle	15	14.7
	Camel	9	8.82
	Small ruminant	54	52.94
	No Animal Abort	24	23.53
Disposal of placental membrane and aborted fetus (N=102)			
	dispose properly	5	4.9
	Throw it on open field	83	81.37
	Report to CAHWS	14	13.72

*Only pastoralist considered, **Only respondents having animals considered.

4.6.1. Knowledge, awareness and practice of the respondents on brucellosis

In table 15, different risk factors such as species of animals owned, history of raw milk consumption, raw meat consumption, knowledge about zoonotic disease specially brucellosis, handling of dystocia case, usage of protective glove and sheltering of domestic animals were considered as important risk factors. Consequently, sheltering of animals, usage of protective gloves and handling of dystocia case were showed significant association with brucellosis seropositivity in humans based on fisher exact test. However, consumption of raw meat, knowledge of other zoonotic diseases and brucellosis didn't stand significantly ($p > 0.05$) with brucellosis seropositivity in human. Even though there is no statistically significant association, all seropositive individuals were consumers of raw milk which elucidated that, raw milk consumption was more associated with brucella infection in the area.

Moreover, the findings of the present study indicated, camel owners were 2.9 times more at risk of brucellosis seropositivity than cattle owner even though it was not statistically significant. This might be due to the fact that, camel owners usually move with their animals far away from their temporary residence in search of water and pasture. Under this condition, they rely only on raw camel milk to feed themselves and assist birth giving cows with the bare hands which are the major predisposing factor of brucellosis infection in pastoral society. In the current study, 90% of the respondent didn't know about brucellosis and it comprise of 10 positive animals and all reactive individuals.

Table 15: Association of knowledge and practice of the society with brucellosis seropositivity in human

Variables	Respondents n (%)	Positive Animals owned n (%)	Positive respondents n (%)	Fisher value	P-value
Animal owned				2.64 ^a	0.208
Camel	44(36.67)	8(3.2)	3(6.82)		
Cattle	50(41.67)	4(2.2)	1(2)		
Other animal species	12(10)	0(0.0)	0(0)		
Have no animal	14(11.67)	0(0.0)	0(0)		
Raw milk consumption				-	1
yes	110(91.7)	12(2.79)	4(3.63)		
No	10(8.3)	0(0)	0(0)		
Raw meat consumption				-	0.572
yes	27(22.5)	3(0.69)	1(3.7)		
No	94(78.33)	9(2.08)	3(3.2)		
Do you know zoonotic diseases:				-	0.318
Yes	34(28.33)	8(1.85)	2(5.88)		
No	86(71.67)	4(0.93)	2(2.32)		
Do you know brucellosis				-	1
yes	12(10)	2(0.46)	0(0.0)		
No	108(90)	10(2.32)	4(3.7)		
Handling of dystocia				6.377 ^b	0.025 [*]
I offer assistance	55(45.83)	3(0.96)	2(3.63)		
Report to CAHWS	53(44.16)	5(1.16)	0(0.0)		
I do nothing	12(10)	4(0.93)	2(12.5)		

Do you use PG during handling dystocia case				-	0.049*
yes	12(10)	2(0.46)	2(16.67)		
No	108(90)	10(2.32)	2(1.85)		
Sheltering of Animals				9.08 ^b	0.025*
All separately	82(68.33)	10(2.32)	1(1.22)		
All sheltered together	2(1.67)	0(0.0)	1(50)		
Some sheltered together	24(20)	2(0.46)	2(8.33)		
No idea	12(10)	0(0)	0(0)		

CAHWS: Community Animal Health workers, ^bfisher exact test value, ^a chi-square value, *Significant.

5. DISCUSSION

Brucellosis is an infectious bacterial disease affecting all domestic and wild animals with significant economic and public health importance. Numerous serological study in Ethiopia indicated that camel and cattle brucellosis is an endemic and widespread disease in different agro-ecological zones of the country ((Mohammed *et al.*, 2011.; Degefu *et al.*, 2011 ; Moti Yohannes *et al.*, 2012 ; Megersa *et al.*, 2011 ; Edao *et al.*, 2018). In the current study, the animal based seroprevalence of camel brucellosis in Amibara district of Afar Region was 7.6% by RBPT and 3.2% by combined RBPT and CFT. Thus, the current study revealed that the overall seroprevalence of camel brucellosis was 3.2% (8/250).

This finding is comparable with the earlier reports of 3.1% in Yabello District of Borena Zone by (Admasu and Kaynata, 2017) , 3.37% in Mehoni district of south eastern Tigray by (Habtamu *et al.*, 2015), 3% in southern lowland of Ethiopia by (Jara *et al.*, 2020), 4.1% in (Aysaita, Dubti and Chifra) districts of Afar region by (Hadush *et al.*, 2013) and 3.1% in Somaliland by (Ghanem *et al.*, 2009). However, it is lower when compared to 5.7% in three camel rearing regions(Afar, somali and borena) by (Teshome *et al.*, 2003), 7.6% in Awash-Fentale and Amibara districts of Afar region by (Zewold and Wereta, 2012), 5.4% in four districts of Afar regional state by (Bekele *et al.*, 2013) and 4.8% in Fafan zone of Somali region by (Mohammoud, 2017).

In camel, the seroprevalence result of the current study was also lower than some reports in other African and middle east countries. For instance, it is lower when compared with a prevalence of 30.5% in Sudan by (Mokhtar *et al.*, 2007), 7.61% in Egypt by (Hassanain and Ahmed, 2012), 19.4% in Jordan by (Dawood, 2008), 11.50% in Egypt by (Sayed *et al.*, 2017) and 37.5% in Sudan by (Omer *et al.*, 2010). The seroprevalence of camel brucellosis appears to follow two distinct patterns: low (2-5%) prevalence in nomadic or extensively kept camels and high (8-15%) prevalence in camels kept intensively or semi-intensively(Abbas and Agab, 2002). As most of camels are kept by pastoralists and few agro-pastoralists in Afar region, sero prevalence of camel brucellosis was low in the current study which agree with the report of (Abbas and Agab, 2002).

In this study, the low prevalence of camel brucellosis might be due to separate sheltering of the camels during the night and low density of camels over a wide range land. Since brucellosis is considered as disease of herd importance, 21.74%(5/23) herd level seroprevalence of camel brucellosis was recorded in the present study which is similar with the reports of (Hadush *et al.*, 2013) in Afar region of North eastern Ethiopia. This finding may be concerned with the number of herds involved in sampling units, presence of high number of camels in the herds and existence of aborted she camels in the herds. In Amibara district, commercial and state-owned cotton farming is a widely known irrigation based agricultural activities by following Awash river. After cotton and all other crops were harvested, different livestock species usually gathered together at Badahmo and Angilale Kebeles to feed on leftover, which facilitated inter species and within herd dissemination of brucellosis.

This may be responsible for comparatively high prevalence of camel brucellosis in Badahmo (7.14%), as infection can easily be transmitted within the herd under this condition, even though there is no statistically significant difference of brucellosis seropositivity between kebeles. In this study, age of the camel was classified as young (≤ 4 years), adult (4-10years) and old (>10) according (Gizaw *et al.*, 2017) and based on the number of parity to see the distribution of diseases in each age group. Accordingly, 34 young, 165 adult and 51 old camels were examined and no reactive animal was found in young age group which might be because of maternal immunity. However, 2(1.21%) and 6(11.764%) of camels were found positive for brucellosis from adult and old age group respectively.

This indicates that higher number of brucellosis seropositive camels were encountered in adults and older animals than in younger camels as it is a disease of sexually matured and pregnant animals which agree with the findings of (Abebe *et al.*, 2017 ; Coelho *et al.*, 2015 ; Megid *et al.*, 2010). Moreover, fisher exact test result show, statistically significant difference of brucellosis seropositivity among different age group($P=0.004$). In animals, sexually maturity and pregnancy favors the occurrence of brucella infection due to increased production of erythritol sugar as the animals become sexually matured specially during pregnancy which enhance the multiplication of pathogen(Gizaw *et al.*, 2017).

The overall sex wise seropositivity was 0% in male camels and 3.43% in female camels. This finding shows no statistically significant difference ($p > 0.05$) in seroprevalence of camel brucellosis among sex groups and it is in agreement with the report by (Teshome *et al.*, 2003 ; Warsame *et al.*, 2012 ; Bekele *et al.*, 2013). However, the number of tested male camels were too small to make comparison and justify that males are less susceptible to brucellosis than females in this study. Similarly, herd size of the camels was considered in this study to see the distribution of the infection in small, medium and large herd group since the probability of contact between animals increases as herd size increases which results in more chance of infection particularly during calving and abortion.

However no statistically significant difference ($p > 0.05$) of brucellosis seropositivity observed in different herd size of camels based on fisher exact test result. Consequently, this finding disagree with the findings of (Gizaw *et al.*, 2017) and (Zewolda and Wereta, 2012) in Afar who reported statistically significant association of camel brucellosis with herd size. Nutrition plays a crucial role in boosting immunity against various infectious diseases. Underfed animals are expected to have poor body condition which is manifested by decreased immunity, recurrent occurrence of infection and susceptibility to non-infectious organisms under normal condition (Kamili *et al.*, 2006 ; Radostits *et al.*, 2007). Therefore, body condition of the camels was considered during this study to see the status of brucellosis in different body condition scores. As a result, statistically significant higher seropositivity of brucellosis was observed in camels with poor body condition score than camels with medium or good body condition score ($P = 0.002$).

Similar findings were also reported by (Abebe *et al.*, 2017) in Ethiopia and (Swai and Schoonman, 2009) in Tanzania. In this study, history of abortion was also considered and showed statistically significant association with brucellosis seropositivity ($p = 0.004$) in that, camel having history abortion were 49.6 times more at risk of brucellosis than those with no history of abortion. This implies that, abortion is the most classical symptoms of brucellosis in camels (Wernery, 2014). So, this finding is in agreement with the report of (Gizaw *et al.*, 2017) in Afar region. Additionally, strong statistical association of abortion and brucellosis sero-positivity in camels might be due to the purposive inclusion of animals with history of abortion for isolation of *Brucella spp* circulating in the study area.

In the present study, it was also indicated that, no camels with history of retained fetal membrane tested positive for brucellosis and statistically there is no association ($P > 0.05$). This might be due to the fact that, camels are supposed to possess diffuse like placenta and are less susceptible to experience placental retention (Fowler *et al.*, 2010) due to brucellosis. By conducting multivariable firth's bias reduced logistic regression analysis, only abortion history has showed significant association with brucellosis seropositivity which also agree with the findings of (Matope *et al.*, 2011).

In Bovine, 10.5% of the total tested animals were found reactive for brucellosis by RBPT among which 2.2% were confirmed to be brucellosis seropositive by combined RBPT and CFT. The current study result is in close agreement with the earlier finding of (Yimer, 2017) who reported 2.4% in Amibara district of Afar region, (Asmare *et al.*, 2007) who reported 2.46% in Sidama zone of southern Ethiopia, (Alemu *et al.*, 2014) who reported 2.0% in Eastern Showa zone, (Kassahun, 2004) who reported 2.5% in Sidama zone of southern Ethiopia and (Jergefa *et al.*, 2009) who reported 2.9% in Central Oromia. However, it is lower when compared to the findings of (Megersa *et al.*, 2011) who reported 10.6% in Borana zone, (Mekonnen *et al.*, 2010) who reported 4.9% in Western Tigray, (Tschopp *et al.*, 2015) who reported 4.8% in pastoral community of Afar and Oromia region adjacent to Awash national park and (Dinka and Chala, 2009) who reported 15.2% in East Showa zone of Oromia region.

Similarly, bovine brucellosis seroprevalence result of the current study was also lower than some reports in other African and Asian countries. For instance, a prevalence of 3.4% in Cameroon by (Awah-Ndukum *et al.*, 2018), 24.5% in Sudan by (Angara *et al.*, 2004), 12-15.8% in Uganda by (Miller *et al.*, 2015) and 26.50% in India by (Chand and Chhabra, 2013) were relatively higher than the present finding. The difference in the seroprevalence between the current and previous study results might be due to variation in sample size used, husbandry and management practices, technical efficiency, virulence of the organisms, diagnostic test employed and coverage of veterinary service. In addition to estimation of seroprevalence, this study was also carried out to assess the risk factors associated with disease occurrence in cattle. In this study, kebeles, sex, age, body condition, parity number, herd size of the cattle and history of placental retention were considered as a risk factor.

Consequently, kebeles, sex, body condition and herd size were not significantly associated with brucellosis seropositivity ($p > 0.05$) whereas the rest showed statistically significant association with brucellosis infection ($p < 0.05$). The insignificant association of sex with brucellosis seropositivity in cattle disagree with findings of (Dinka and Chala, 2009 ; Kebede *et al.*, 2008), who reported higher cases of brucellosis in female animals than in males. However, the number of male animals included into the current study were too small to make comparison and justify that male cattle are less susceptible to brucellosis than female. Concerned with body condition score, 3.57% of animals with good body condition were test positive for brucellosis whereas no reactor animals encountered from animals with poor body condition.

Fortunately, this finding is in agreement with the report of (Awah-Ndukum *et al.*, 2018), who stated insignificant association of body condition score with susceptibility to brucellosis. Similarly, herd size of the cattle was considered as risk factors in this study since brucellosis is a disease of herd importance. Based on this, the herd level seroprevalence of bovine brucellosis in the present study was 18.18% which is in close agreement with the finding of (Ibrahim *et al.*, 2010). Even though large herd sizes are more prone to brucella infection, no significant difference of brucellosis infection observed among different herd size in the current study ($P > 0.05$). Consequently, this finding disagrees with findings of (Tolosa, 2004). Based on fisher exact test result, age, number of parities, history of abortion and placental retention were considered and showed statistically significant association with brucellosis seropositivity in cattle ($P < 0.05$).

However, after computing firth bias reduced logistic regression analysis, only multiparous and animals with history of abortion showed statistically significant association with brucellosis seropositivity ($p < 0.05$). This finding is in agreement with (Alemu *et al.*, 2014) and (Isloor *et al.*, 1998) who reported significant association of parity and abortion history with brucellosis in cattle. This might be due to the fact that, multiparous animals usually stay in the herd for a long period of time for breeding purpose and had greater chance of being exposed to carrier animals.

Human brucellosis is a widespread disease in pastoral areas of different African and Asian countries (Abbas and Agab, 2002). In the present study, an overall human brucellosis seropositivity of 3.33% was identified by combined RBPT and CFT. This finding is fairly in agreement with the findings of (Tolosa *et al.*, 2007) in Jimma University hospital, (Kassahun *et al.*, 2006) in Addis Ababa, (Eshetu *et al.*, 2018) in Eastern Ethiopia and (Tibesso *et al.*, 2014) in Adami Tullu who reported 3.6%, 4.8%, 4.8% and 2.15% respectively. However, the result of the present study is lower than the finding of (Vancelik *et al.*, 2008) in Turkey, (Sharma *et al.*, 2016) in India, (Aworh *et al.*, 2013) in Nigeria, (Rasul and Mansoor, 2012) in Erbil city of Iraq, (Workalemahu *et al.*, 2017) in southern Ethiopia, (Baba *et al.*, 2001) in eastern Nigeria, (Zewold and Wereta, 2012) in selected Afar districts and (Alkahtani *et al.*, 2020) in Saud Arabia who reported 5.4%, 4.96%, 24.1%, 10.7%, 10.6%, 5.2%, 15% and 12.8% respectively.

On the other hand, the present study result is higher than the finding of (Rahman *et al.*, 2016) who reported 2.0% in Bangladesh, (Haileselassie *et al.*, 2011) who reported 1.2% in western Tigray and (Michael *et al.*, 2016) who reported 0.0% in Jimma town and Chora Botor district. In pastoral and agro-pastoral area, prevalence of brucellosis in human is greatly influenced by status of the disease in animals (Omer *et al.*, 2002). Brucellosis occurrence fluctuates extensively, not only between countries but also within a country (Khan and Zahoor, 2018). So, the possible justification for the variation of human brucellosis seroprevalence in the current study was due to difference in the sample size, difference in the life style of the society, type of diagnostic protocol employed and socio-economic status of the study population. In the current study, environmental and demographic risk factors such as kebele, gender, age groups, educational level, occupation, marital status and family size were considered and only occupation found significantly associated with brucellosis seropositivity in human.

One research work conducted in Malaysia indicated that, prevalence of brucellosis is more common in males ranging from 20-45 years old (Tay *et al.*, 2015). This showed that, males are at greater risk of contracting brucellosis since they commonly involved in the handling of livestock and consume uncooked animal product specially in pastoral area. However, the finding of the current study indicated that, gender is not significantly associated with brucellosis seropositivity and didn't much with the report of the above research finding in Malaysia.

This may be due to small sample size of the present study and shared responsibility among male and female of Afar pastoralist. Study conducted by Boschioli *et al.*, (2001) indicated, in rural area where women handle livestock, the incidence rate of brucellosis is elevated in females. Concerned with the occupational hazard of brucellosis, the disease is commonly found in herdsmen, people working in the dairy or meat industry, veterinarians, and laboratory professionals (Khan and Zahoor, 2018). Consequently, it was also attempted to include peoples with different occupation to observe the status of the disease in peoples with different work type such as pastoralists, government workers, students and peoples who rely on daily based wage. As a result, only 2.22% (2/90) of pastoralist and 25% (2/8) of the study participants with non-permanent job were found positive for brucellosis which showed statistically significant association of occupation with brucellosis seropositivity ($p < 0.05$) in humans.

Briefly, greater percentage of participant with non-permanent and daily based wage work type were tested positive for brucellosis than others in the present study. It was also indicated that, individuals with non-permanent work were 18.8 times more at risk of contracting brucellosis when compared with governmental employee and also showed statistically significant association ($P = 0.029^*$, 95% CI: 1.324, 2730.32) with brucellosis infection. This might be due to fact that, some of these peoples were the family members of pastoralists whom own animals and also run daily based wage work to increase their income. Due to this, they may become more exposed to brucellosis infected animals since they frequently visit the village of their family and consume raw milk from different household.

More than 90% of peoples in Afar region rely on pastoral activity and they spend almost all their entire life with their animals. The main source of their food is also animals' origin specially milk which is commonly consumed without boiling. In the current study, 96.4% of the respondents drink raw milk which agree with the findings of (Ntirandekura *et al.*, 2018b) who reported drinking unpasteurized milk and eating non-inspected meat to be among possible factors which could contribute to transmission of brucellosis in humans. So, considering the low awareness level of the society and poor infrastructure in the area, pastoralists are highly vulnerable to this neglected infectious zoonotic disease through practicing this non safe activity.

In the current study, the number of tested individuals from each demographic risk factor specially gender, occupation and educational status was not sampled proportionally. Due to this, it is impossible to reach at the final conclusion of non-significant association of some risk factors with brucellosis seropositivity in human. Study conducted in Saudi Arabia shows, brucellosis prevalence is common in people of the age group 13-40 years and get decreases in the older age group(Makita *et al.*, 2008). In this study, age of the respondents was also one of the considered risk factors to evaluate the status of the disease in different age group. Amazingly, 5.71% (2/35) seropositive peoples were aged above 60 years where as 2.35% (2/85) of them were in 13-59 age group. This agree with the finding of (Makita *et al.*, 2008) who reported increased vulnerability of aged peoples to brucellosis.

Brucellosis is transmitted to humans through consumption of unpasteurized dairy products or through direct contact with infected animals, placentas or aborted fetuses (Haileselassie *et al.*, 2011). Evidences shown that, the social habit of raw milk and meat consumption, unsafe handling of aborted fetuses and placenta, assisting parturition, and occupations related to animal contacts have been reported to be some important epidemiological factors for human brucellosis(Dinka and Chala, 2009; Haileselassie *et al.*, 2011).

In the current study, participants from Halaydge kebele were 6.964 times and those from Werer kebele were 2.27 times more at risk of contracting brucellosis when compared with participant from Angilale kebele. This might be due to the fact that, majority of the Amibara district pastoralists usually prefer and migrate to Halaydge national park during the period of drought season to look for pasture and water and move back to Werer kebele together to feed their animals upon the leftover of cotton farm. This condition allows close contact of different animal species and herds with unknown health history which create a favorable condition for ease transmission of brucellosis among different livestock species and later to their owners through drinking raw milk, consumption of uncooked meat and contact with aborted fetal materials.

Ethiopia is one of the Sub-Saharan countries highly endemic to malaria, although there are many malaria-like diseases in the country. For instance, brucellosis is one of these group of diseases which is manifested by clinical pictures common for malaria and it is also endemic in Ethiopia (Eshetu *et al.*, 2018). In the current study area, typhoid fever and malaria were the top recorded cases due to the existence of numerous swampy area which is associated with active irrigation activity. So, people's living in the Amibara district periodically visit hospital and health stations to seek treatment for malaria and other infectious diseases. Since the clinical picture of brucellosis seems like that of malaria and typhoid fever and 3.33% human brucellosis seroprevalence was recorded in the current study, there is a high probability of misdiagnosing brucellosis as either malaria or typhoid fever which is also reported by (Ntirandekura *et al.*, 2018b).

Misdiagnosis of brucellosis may be due to lack of awareness about the disease by medical staff, limited diagnostic facilities, lack of experience among laboratory personnel and nonspecific clinical picture of the disease (Kunda *et al.*, 2005). It is obvious that, education is the only key factor to enhances the knowledge, awareness level and to modify the lifestyle of the society. In pastoral areas of developing countries, the access to basic infrastructure such as education, road, electricity and health service is not fulfilled due low economy of the countries and mobile life style of the society. To evaluate the effect of education on the knowledge and awareness level of the society about brucellosis, academic status of the respondent was considered as an important risk factor. Meanwhile, non-significant association of educational background with brucellosis seropositivity of the respondent encountered in the current study($p>0.05$).

A study conducted in Kenya showed a high level of knowledge of brucellosis in pastoral communities where respondents reported brucellosis to be a zoonotic disease and abortion as its common symptom (Obonyo and Gufu, 2015). On the contrary, study conducted by Tesfaye *et al.*, (2013) in Ethiopia showed, zero knowledge of the respondent about zoonotic importance of brucellosis. So, this may be due to the difference in the educational access and coverage in pastoral area of the two countries. In addition, marital status and family size were also considered and showed non-significant association with seroprevalence of the disease in human.

From a total of 120 respondent interviewed, 36.67% were camel owners and 41.67% were cattle owners. The average number of animals owned by each respondent is (47.08 ± 4) . In this study, 91.7% of the respondent drink raw camel or cattle milk whereas 8.3% of them responded that, they drink milk only after boiling. Even though, association of raw milk consumption and brucella seropositivity is statistically insignificant, all of the seropositive individuals were consumers of raw milk. Based on descriptive statistical analysis, 78.33% (n=94) of the respondents told that, they do not consume raw meat which is mainly concerned with cultural issue. Consumption of raw meat is considered as “*Haram*” among Afar pastoralist. However, all of the seropositive participants were not raw meat consumers. This indicates that, the rate of brucellosis infection in the current study area was greatly associated with drinking of raw milk and contact with infected animals than consumption of uncooked meat.

Similarly, about 45(37.5%) of the respondents were used to slaughter animals at home to fulfil their meat demand among which 2(4.44%) of them were confirmed to be brucellosis seropositive. This may be due to poor hygiene, cross contamination of cooked and raw food and inappropriate food handling among pastoralists of Afar region. It may be also associated with slaughtering of carrier animals, very poor habit of cleaning environment and equipment’s, accidental self-cutting and exposure of synovial fluid with high load of brucella organisms to the open environment which facilitate widespread occurrence of the disease. On the other hand, about 54(45%) use traditional medicine to treat wounds on their hands which allow brucellosis infection to enter into their body through this broken skin.

During handling of dystocia cases, majority of the respondents (98.2%) reported that they used hand pulling techniques without using protective gloves (90%). Study conduct by (Eshetu *et al.*, 2018) indicated , brucellosis were 5.11 times more in those who had assist animals during parturition compared to those who did not. Protective gloves for use during assistance of parturition could not be available in pastoral areas. In the current questionnaire survey, majority of the respondent 78(76.47%) told that, their cattle/camel will get mixed with other domestic and non-predator wild animals during grazing on the field. So, this condition created a favorable situation for widespread and permanent occurrence and circulation of brucellosis in the area by sharing grazing area, water point and direct contact with infective materials.

About 80% of the respondent don't think that disease can get transmitted from wild to domestic animals. On the other hand, 80(88.89%) of the respondent insist that, they keep camels and cattle's in Halayde national park to look for water and pasture which allow close contact of domestic animals with jungle beast. Due to this, brucellosis can spread in either direction from contaminated pasture and water point or by direct contact with aborted fetal materials. Study conducted in Tanzania indicated, the interactions between wildlife and livestock as potential risk for brucellosis transmission to humans and livestock(Ntirandekura *et al.*, 2018b). Concerned with sheltering of livestock, about 82(68.33%) of them responded, they shelter their animals separately during the night because of the aggressive behaviors of the camels toward other animals.

In this study 66.67% of the respondents complained frequent occurrence of abortion in their herds while small ruminants were mainly associated with the reported reproductive disorder. Concerned with this situation, camel was the least reported animal to experience abortion in the herd 5(4.49%). Even though camels were the least reported animals with abortion case, they were at greater risk of contracting brucellosis from other latent carrier and aborted domestic animals. Regarding the management of aborted fetal membrane/aborted fetus and discharge, 83(81.37%) of the respondent told that, they throw it on the field.

Additionally, it was also encountered that, pastoralist leave died camel calf at home to show the mother she camel every morning by considering that, the activity will instinct milk letdown. So, these activities can be the major predisposing factors for widespread occurrence of brucellosis both in camels, other domestic animals and the owners. However, 4.9% of the respondent practiced proper disposal of the aborted fetal materials. In this study, about 90% of the respondent had never heard about brucellosis and 65% of the them need to acquire detailed information about it. Finally, the major symptoms of brucellosis were reported to respondents and about 99.1% of them were interested in giving blood samples for screening of brucellosis.

6. CONCLUSION AND RECOMMENDATIONS

Brucellosis is one of the most important bacterial diseases of domestic and wild animals with significant economic and public health importance. Both animals and human can contract brucellosis through direct contact with infected animals and their excreta, ingestion of infected materials and sometimes through aerosol transmission. Based on the nature of the disease and ease of transmission, the pastoral society of Africa and central Asia are at great risk due to their habit of consumption of raw milk and close physical contact with animals. A cross-sectional study was carried out from October 2019 to May 2020 with the objectives of determining seroprevalence of brucellosis in camels, cattle's and the owners of sampled animals including interested individuals in Amibara district of Afar region, Ethiopia. As a result, low seroprevalence of camel (3.2%) and cattle (2.2%) brucellosis was indicated in the current study. The herd level seroprevalence of brucellosis was 21.74% and 18.18% in camel and cattle respectively.

In the current study, the overall seroprevalence of human brucellosis was 3.33% by combined RBPT and CFT. Based on multivariate firth's bias reduced logistic regression, only history of abortion showed significant association with brucellosis seropositivity in camel. In cattle, multi parity and history of abortion showed statistically significant association with brucellosis based on firth's bias reduced logistic regression. In humans, different socio-demographic and environmental risk factors were considered. As a result, occupation, handling of dystocia cases, usage of protective gloves and sheltering of animals showed significant association with brucellosis seropositivity. Based on descriptive statistical test, all seropositive individuals were raw milk consumer.

In questionnaire survey it was indicated that, majority of the respondent practice common risk factors supposed to expose them to the disease due to their poor knowledge and the nature of their life style. Even though, most of the considered risk factors didn't show statistically significant association with brucellosis seroprevalence in humans, peoples are practicing the common risk factors and the existence of disease has confirmed in both animals and human. In general, the current study provided baseline data on camel, cattle's and human brucellosis in the current study district of Afar region, Ethiopia. It was indicated that, the pastoral society are at great risk of

contracting brucellosis at any time and situation due to lack of knowledge on zoonoses, traditional husbandry, poor management practices, consumption of raw milk and handling of birth giving cow and aborted materials with bare hand together with possessing of seropositive animals.

Therefore, based on the present finding, the following recommendations are worth mentioning:

- A comprehensive active assessment and surveillance studies are required to understand the distribution of brucellosis and its transmission dynamics at domestic-wild life interface and its zoonotic significance.
- Working to control risk factors, establishing brucella diagnostic service in human clinics and hospitals and implementation of One health approach framework to attain optimal health for people and animals.
- Enhancing the awareness level of the pastoral society about the public health and economic importance of brucellosis through training and workshop. For instance, informing not to touch aborted fetal material without using protective wearing's in addition to abstaining themselves from drinking raw milk.
- Detailed studies on isolation and characterization of circulating strain and biotypes in the area need to be conducted.

7. CHALLENGE AND LIMITATION OF THE STUDY

In the current study, isolation of *Brucella spp* was attempted from cattle and camels with history of abortion. Out of 24(15 vaginal swab and 9 hygroma) clinical samples cultured at Aklilu Lemma Institute of Pathobiology (ALIPB), two samples showed probable and unconfirmed brucella colony. But, the unexpected occurrence of Covid-19 pandemic during this laboratory phase prevent me not to conduct biochemical test and molecular detection of the colony. So, the suspected colony was preserved (Annex 5) and the remaining biochemical and molecular tests are supposed to continue after the issue of this outbreak is resolved.

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9. ANNEXES

Annex 1: Pictures of camel; cattle's and human during sample collection at field and health station





Annex 2: Pictures during serum separation from whole blood sample of camels, cattles and humans





Annex 3: Pictures during questionnaire survey on public health importance of brucellosis



Annex 4: Laboratory Procedures

A. Rose Bengal Plate Test

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

Then 30 μ l of serum sample was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.

The antigen and serum were mixed thoroughly using a micro pipette tip (a tip being used only once)

3. The plates were Rocked by hand for about 4 minutes
4. Finally each plate was examined for agglutination in a good light
5. Magnifying glass was used when micro agglutination is suspected.

Interpretation

0 = no agglutination

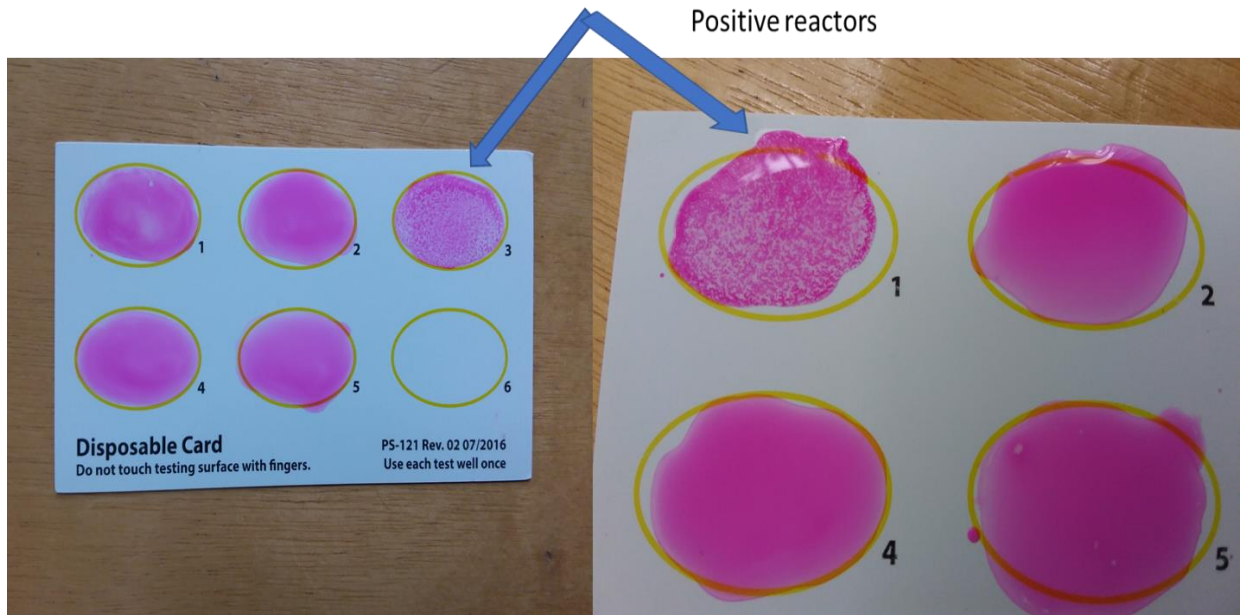
+ = barely perceptible

++ = fine agglutination, some clearing

+++ = coarse clumping, definite clearing

Those samples identified with no agglutination were recorded as negative those with +, ++, +++, were recorded as positive.





B. Complement Fixation Test procedure

Principle

The complement system consists of series of proteins that, if triggered by an antigen - antibody complex react in a sequential manner to cause cell lysis. The test has two steps, in the first step antigen, test serum and complement are mixed and incubated and in the second step an indicator system which consists of sheep red blood cells (SRBC) and Amboceptor (that sensitizes RBC to the action of complement) is added. If the test serum contains antibodies to *Brucella* an antigen-antibody complex is formed (positive reactions) and the complement is used up, so it cannot react in the hemolytic system. Therefore, no lysis of SRBC will occur and SRBC will remain intact. If the test serum does not contain *Brucella* antibodies (negative reaction) complement will not be fixed and lysis of RBC will occur. In indicator system SRBC and Amboceptor forms immune complex. In the positive test sample SRBC-amboceptor complex remains unaffected resulting in sedimentation of the RBC. The negative test sample has unbound complement that will bind to RBC- amboceptor immune complex this binding to the erythrocyte surface will disrupt the erythrocytes and hemolysis will occur.

Reagents

1. Complement
2. Hemolytic serum (Amboceptor)
3. Hemolytic system
4. *Brucella* antigen
5. Sheep Red Blood cells (SRBC)
6. *Brucella abortus* positive control serum
7. Negative reference serum
8. CFT buffer- veronal calcium magnesium (VCM)
9. Alsever's solution

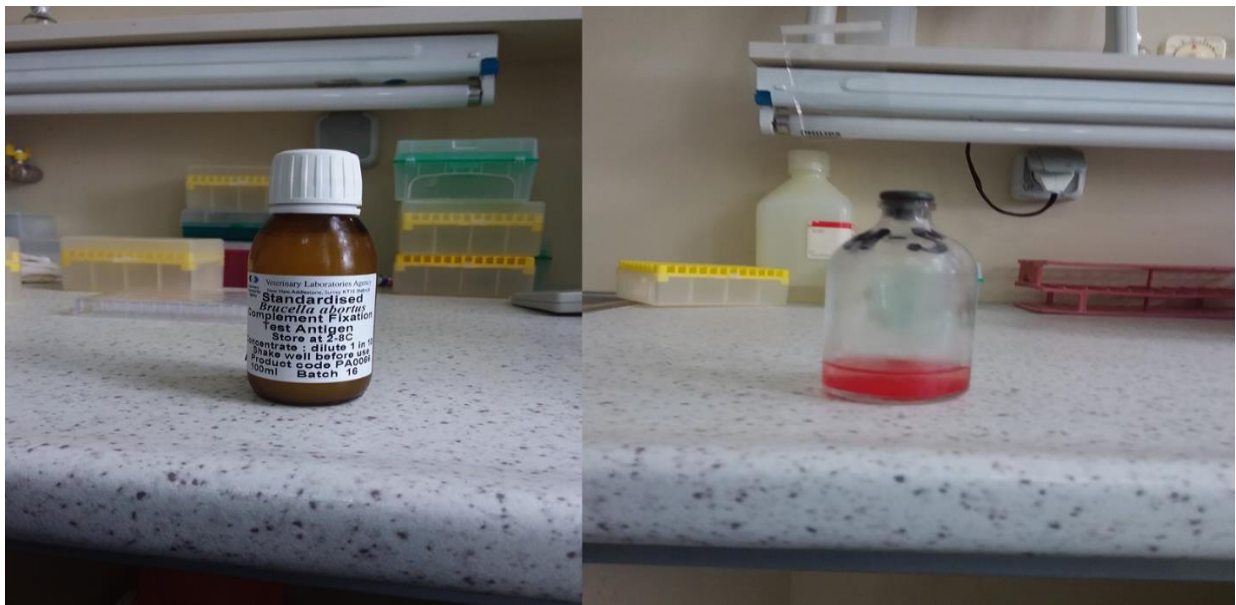
Procedure

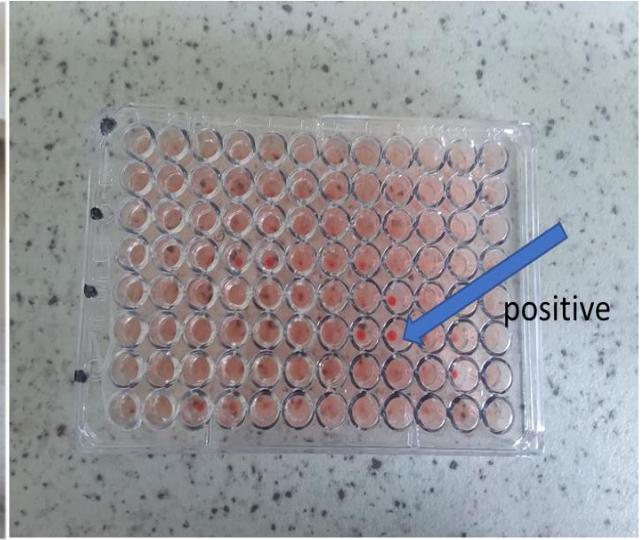
1. Test sera and appropriate working standards are diluted with an equal volume of veronal buffered saline in small tubes and incubated at 58°C (+/-2°C) for 30 minutes in order to inactivate the native complement.
2. A U-shaped 96-well microtiter plate are prepared
3. Column 1 through 12 is used for test serum
4. Each well of row "A" is used for anti-complementary control
5. 25 µL of VCM is dispensed by using a hand held 12 channel micropipettes into wells of rows A-H
6. Using standard 96-well U-bottom microtiter plates, 25 µL volumes of diluted test serum are placed in the wells of the A, B and C rows and homogenize wells of row C (column 1-12) of the test plate and pick up 25 µL from row C of the test plate and deliver to the wells of row D (column 1-12) .The serial dilution is continued to row H (column 1-12) from which after homogenization 25 µL is picked up and discarded.
 - a. 25 µL of diluted antigen is added into all the wells of rows B-H and wells antigen and positive and negative control wells.

- b. 25 μ L of diluted complement is added into the wells of rows A-H and all controls wells except hemolytic system.
 - c. The plate is covered with sealer and incubated at +37°C under constant agitation on incubator shaker for 30 minutes.
 - d. 25 μ L of hemolytic system is added to all wells including control wells.
 - e. The plate is covered with sealer and incubated at +37°C under constant agitation on incubator shaker for 30 minutes.
7. Control wells containing: Positive serum +diluent +Complement +Ag +HS, negative serum + complement + diluent+Ag +HS, antigen + complement + diluent +HS, HS + diluent and complement + diluent +HS, are set up to contain 75 μ l total volumes in each case.
8. The results are read after the plates have been left to stand at 4°C for up to 1 hour to allow unlysed cells to settle.

Interpretation

Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative





Annex 5: Unconfirmed *Brucella* colony



Annex 6: Ethical clearance Certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/03/01/12/2020

Name of Applicant: **Fekadu Gutema Wegi (DVM, MVSc fellow)**

Address: College of Veterinary Medicine and Agriculture (Addis Ababa University)

Title of the project: *Sero-prevalence and isolation of Brucella species from cattle and camel with history of abortion and their public health significance in selected districts of Afar Regional State, Ethiopia*

Date of application: **15/10/2019**

Nature of the project: **mildly invasive**
Target animal species: **cattle and camels**
Number of animals involved: **based on the number of abortion cases**
Study area: **Afar Regional State, Ethiopia**

Minutes No. and date of review: **VM/ERC/01/12/020, 03/01/2020**

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 this is deemed necessary
3. A separate clearance is required for any work (except questionnaire) on human subjects

Dr Getachew Terefe
Chairman


Signature



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

ፋክስ } ስልክ } ፖ.ሣ.ቁ } ቢሾፍቲ/ደብረዘይት-ኢትዮጵያ
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Annex 7: Sample collection format

Region _____ Zone _____ District _____ PA _____ Date _____ Herd
size _____

SN	Code	Spp	Sex	Age	Owner name	Type of sample taken					Breeding history of sampled animal			
						Blood	Milk	A.fetus	v. swab	Hygroma	Parity.N	Abortion	Still. birth	RFM
1														
2														
3														
4														
5														
.														
.														

RFM=Retained Fetal Membrane, Parity.N =Parity Number, St. Birth= Still Birth, V.swab= Vaginal swab, Bld=blood, Mlk=Milk, A.fetus=Aborted fetus

Annex 8: Questionnaire format used to investigate knowledge, attitude and practice of the society about brucellosis.

I. General information

District_____ Kebele_____ PA_____

Name _____ Age _____ sex _____

Occupation: a. pastoralist b. agro pastoralist c. trader d. other

Educational status: A-illiterate B. read and write C. elementary D. high school E-others

Marriage: A. single B. married

Family size (if married): _____

II-Information related to livestock keeping practice in study area

1-Do you have animals (cattle, sheep, goat, camel)?

A. yes B. no

2-If yes, please specify the animal species you have and their number.

S. N	Animal species	Quantity	Remark
1	Cattle		
2	Camel		
3	Sheep		
4	Goat		
5	Others		

II-Information related to importance of keeping livestock and usage of their product

1-For what purpose do you keep animals (more than one answers are possible)

A-For generating income by fattening and selling

B-For generation of income by selling milk and milk product

C-To slaughter and eat meat

D-To have good social status

2-Do you consume raw milk?

A. yes B.no

3- Do you consume raw meat?

A. yes B.no

4. If yes, from where and how do you get meat?

A-Slaughter at home

B-Slaughter at village and share it between group(kircha)

C-From butcher house

E-From different source

5-Do you think that disease can be transmitted from cattle/camel to human either through consumption of raw milk or meat?

A-yes

B-No

6-For question No-6 if your answer is yes, please, specify those diseases you know

A. _____

B. _____

C. _____

D. _____

E. _____

7-If your hand or other parts of your body get wounded unfortunately, what do you do?

A-I live as it is until it gets heal by itself B-I go to clinic to look for therapy C- I use traditional treatment

III-Information related to livestock Management and Health care

1. If you have different composition of livestock how do you herd them?

A- Separately B-mixed on the field during grazing/browsing C -get mixed at watering point.

2-At night how do you shelter the different species of livestock you have?

A-All separately B-some share common shelter C-All share common shelter

3-For question No 2, specify those animal species who share common shelter.

4-Do you allow your animals to graze close to or inside national park?

A-yes

B-no

5-Do you think that your animal can get sick after having contact with non-predator wild animals?

A-yes

B-no

6- If your pregnant cows get suffer during birth giving what do you do?

A-Report to CAHWS B-I will offer assistance by myself C- I do nothing

7-For question number 6 if your answer is B, how do you do it?

A-Hand pulling

B-others

8-For question number 6, if your answer is B, do you wear any protective while helping the cow?

A-yes

B-no

10-Do you wear any protectives during care giving for the new born?

A-yes

B-no

11-If you see died newborn calf in your village or on the road what do you do?

A- I bury it

B-I burn it

C-report to CAHW

D-I leave to be cleaned by scavenger

Iv-Information related to knowledge and attitude of the society about brucellosis

1-Have you ever heard about the disease called brucellosis?

A-yes

B-no

2- 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

6- Do want to have more information about brucellosis?

A-Yes

b-No

8-Are you volunteer to get tested for this disease?

A-yes

B-No