JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

SEROPREVALENCEOF BOVINE BRUCELLOSIS AND ITS ASSOCIATED RISK FACTORSAND KNOWLEDGE, ATTITUDE AND PRACTICE OF CATTLE OWNERS TOWARDS THE DISEASE IN GAMBELLA AND ITANG DISTRICTS OF GAMBELLA REGION, SOUTHWESTERN ETHIOPIA

BY

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MSc. THESIS

MARCH 2020

JIMMA ETHIOPIA

Jimma University
College of Agriculture and Veterinary Medicine
School of Veterinary Medicine

Seroprevalence of Bovine Brucellosis and its associated risk factors and knowledge, attitude and practice of cattle owners towards the disease in Gambella and Itang special districts of Gambella region, south western Ethiopia

By

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The Thesis Submitted to Jimma University College of Agriculture and Veterinary Medicine, in partial fulfillment of Masters of Science (M.Sc.) in Veterinary Public health

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March2020
Jimma, Ethiopia

DEDICATION

This paper is dedicated: to my mother, Birke Barkessa and my father Alemayehu Kassaye, who never went to school themselves for raising us up and sending me to school and supporting me financially and morally throughout my academic career. To my sister Alganesh Alemayehu who melts her life as a candle for us, I never forget her and to my wife, Roza Ashebir, and my kids; Amanuel and Jinanus Abiyot for their all rounded support, without their permission I wouldn't be here today.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my bonafide work and that all sources of material used for this thesis has been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVPH) degree at Jimma University, College of Agriculture and Veterinary Medicine 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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BIOGRAPHICAL SKETCH

The author was born in Anfillo woreda, Kellem Wollega Zone, Oromia regional State on October 20, 1980 G.C from his Mother Birke Barkessa and from his father Alemayehu Kassaye. He attended his primary school at Shebel Primary school at Shebel kebele, Junior Secondary School at Ras Gobena and secondary school at Gambella high school at Gambella Town. In 1993 he joined Agarfa Multipurpose Agriculture Training Center and graduated with certificate in animal health technician. Soon after, he hired as Animal Health Technician in 1994 E.C at Gambella Woreda Agriculture Office. In 1997 he joined Alage ATVET College and graduated with diploma in animal health in 1999. After graduation he served as an Animal Health Assistant and Senior Meat Inspector. In 2001 E.C, he has joined Haramaya University and graduated with a Bachelor degree in veterinary Science in 2005.Then after he joined Gambella Agriculture Research Institute and served as animal health researcher since 2006. He again has joined graduate program at Jimma University to pursue his career in Veterinary Public Health.

ACKNOWLEDGEMENTS

Above all, thanks to my almighty God for his help in giving me courage to cop up complicated situations I faced for pursuing my study and for his help and courage during my whole study time. I would like to express my deepest and sincere gratitude to my academic advisors Professor Tadele Tolosa, Dr. Mukarim Abdurhaman (JU) and Dr. Biniam Tadesse (NAHDIC) for their overall research guidance and taking their time to correct this manuscript.

My special thanks goes to National Animal Health Diagnostic and Investigation center staff members especially Dr. Fasil Akililu, Dr. Biniam Tadesse and Dr. Getachew Tuli, for their positive cooperation during sample preservation and laboratory processing. Again, I want to express my deepest gratitude and appreciation to my staffs, Mr Tesfamikael Fiseha, Yonas Kefiyalew (PhD), Zerihun Belachew, Gatwich Tang, Likneh Teshome and Fitsum Tesfaye for their positive cooperation and field guidance during sample collection from the Districts.

I would like to appreciate the cooperation of Gambella and Itang special District Agricultural and Rural Development coordinating office for facilitating the fieldwork. Finally, my heartfelt gratitude goes to my Wife Roza Ashebir, my Sister Feven Alemayehu and Nahusenah Alemayehu for their financial and moral encourages to accomplish this study once again I thank you.

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LIST OF ABBREVIATIONS

CFT	Complement Fixation Test
CHE	Central Highlands of Ethiopia
CI	Confidence Interval
CSA	Central Statistical Agency
CSF	Chaffa State Farm
^{0}C	Degree Celsius
FPA	Fluorescence polarization assay
GRAFDA	Gambella region Animal and Fishery Development Agency
MoA	Ministry of Agriculture
NAHDIC	National Animal Health Diagnostic and Investigation Center
OR	Odd Ratio
PAHO	Pan American Health Organization
PCR	Polymerase Chain Reaction
P-value	Probability value
RBPT	Rose Bengal Plate Test
RFM	Retained Fetal Membrane
S19	Strain 19
SPSS	Statistical Package for Social Science
USDA	United States of Development Agency

ABSTRACT

Brucellosis is economically important zoonotic bacterial disease caused by genus Brucella. A cross-sectional study was conducted on cattle in Gambella and Itang districts Gambella regional state between February 2019 and November 2019 to assess bovine brucellosis seroprevalence, potential risk factors, knowledge-attitude and practice of cattle owners about brucellosis. The study districts were selected purposively. However, peasant association, herd and individual animals were selected randomly. A total of 400 blood samples were collected from local breed cattle of above six months of age. The RBPT screened 19 *Brucella* seropositive out of 400 (4.75%) (95%CI 1.04-8.05) and positive sera were further retested by using CFT and the combined result (RBPT and CFT tests) 8 (2%) (95% CI: 0.75-3.2) sera were confirmed seropositive. Out of 80 herds included in the study, 6(7.5%) (95% CI: 4.6-17.2) were seropositive using CFT with at least one seropositive animal in the herd. The overall seroprevalence of brucellosis was 2% and 7.5% at animal and herd level respectively. Moreover, information was gathered on individual animal and herd to assess risk factors using a semi- structured questionnaire prepared for this purpose. The result of multivariable logistic regression analysis showed that herd size (OR: 9.481, 95%CI**:** 1.09-82.48,p=0.041),history of previous abortion (OR: 7.8, 95%CI: 5.75-12.38, P=0.003)and history of retain fetal membrane (OR: 32.18: 95%CI: 3.78-27.38, P=0.001) were foundassociated for *Brucella* seropositivity. The results of questionnaire survey revealed that the majority(87.5%) of respondents do not have sufficient knowledge about brucellosis and its risk factors, about 93.75% of the have the habit of consumption of raw milk and 81.25% of respondents were assisting parturition without glovewhich put themat high risk of acquiring the infection. Although the overall prevalence of bovine brucellosis was low in study area, it could serve as source of infection to different herds as there were foci of infection in herds and brucellosis is highly contagious disease. Hence, avoid raw milk consumption, increasing awareness creation, deep burring of aborted fetuses and fetal membrane measures should be implemented to reduce risk of infection and transmission of the disease in livestock and human in the study area.

Key-words: *Bovine, Brucellosis,Risk factors, Seroprevalence, Gambella, Ethiopia*

1.INTRODUCTION

1.1. Background

Ethiopia has one of the largest livestock populations in Africa, which consists of59.5 million cattle, 60.9 million small ruminants, about 1.21 million camels and 11.01 million equine and 59.5 million poultry(CSA, 2016/2017). It contributes more than 30% of the agricultural gross domestic product and 19% in export earnings (MoA, 2012). This sector represents a major national resource and form an integral part of the agricultural production system(IFPRI, 2006; Lobago *et al.,* 2006). Even though it has significant contribution to the economy, the comparatively huge livestock resources of the country the economic return gained from this subsector do not coincide; because of they were affected by differentinfectiousdiseases which greatly affect the economy and public health (Yifat *et al.,* 2012). Among these diseases brucellosis is one of the major diseases affecting the dairy industry responsible for low productivity.

Brucellosis is an infectious bacterial disease caused by genus Brucella (Hirsh and Zee, 1999), which are Gram-negative, facultative, intracellular coccobacillary comprised of species based upon biochemical features and their correlation with preferred host species (OIE, 2000).Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B.melitensis* and rarely by *B. suis*, is characterized by late term abortion, infertility and reduced milk production (OIE 2008). Aborted foetuses and discharges contain large number of infectious organisms and transmit the disease within and in between herds. In addition, chronically infected cattle can shed lower numbers of organisms via milk and reproductive tract discharges and can also vertically transmit infection to subsequently born calves and maintain disease transmission (McDermott and Arimi 2002). Animal susceptibility to brucellosis depends on their natural resistance, age, sex, level of immunity and environmental Stress (Radostits, 2000).

There are a lot of factors that influences the epidemiology of cattle brucellosis including factors associated with disease transmission between herds, factors influencing the maintenance and spread of infection within herds (Crawfordet al. 1990). In order to setup the proper strategy for the disease control and prevention measures knowing the epidemiology of brucellosis is crucial, however, such information is inadequate in sub-

Saharan Africa. Consequently, appropriate preventive measures have not been undertaken in this part of the world (McDermott and Arimi 2002).

The prevalence is highest in the Mediterranean countries, Central and South America, the Middle East and South Asia (Alballa R.S, 1995). Some of the reasons for this may be due to endemicity ofthe disease in the area, huge small ruminant population, existences of risk factors and lack of control strategies in the areas. Although the disease has been eradicated from most of the developed countries, it is still a major public and animal health problem in many developing countries, where livestock are a major source of food and income (Pappas et al., 2006). The high prevalence is probably due to the fact that many countries have not yet started control or eradication schemes (Alveraz *et al.,* 2011).

In Africa, bovine brucellosis was first recorded in Zimbabwe (1906), Kenya (1914) and in Orange Free State of South Africa in the year 1915 (Chukuwu, 1985). However,still the epidemiology of the disease in livestock and humans as well as appropriate preventive measures are not well understood and such information is inadequate particularly in sub-Saharan Africa. The surveillance and control of brucellosis in this region is rarely implemented outside South Africa (McDermott *et al*., 2002). In dairy production, the disease is a major obstacle to the importation of high yielding breeds and represents a significant constraint to the improvement of milk production through cross breeding (Mustefa and Nicoletti, 1993).

In Ethiopia, the rural people are mainly dependent on livestock and their relationship with them is very close. Moreover, people often consume raw animal products (Ameni and Erkihun, 2007). The high prevalence is probably due to the fact that many countries have not yet started control or eradication schemes (Alveraz *et al*., 2011). Brucellosis is endemic in Ethiopia since 1970 (Yohannis, 2017). Since then, studies have demonstrated the presence of antibodies against Brucella in animals and humans in different parts of the country (Yohannes *et al.,* 2013; Degefa *et al.,* 2011; Ibrahim *et al*., 2010; Megersa *etal.,* 2000).

1.2. Statement of the problem

Livestock provides a lifeline for a large proportion of 95% of the world's rural population

that live in the developing world (Wadood *et al*., 2009). Brucellosis has impose significant impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Maadi *et al*., 2011). It causes losses due to abortion or breeding failure in the affected animal population, diminished milk production and causing reduced work capacity through sickness of the affected human (Bashitu *et al.*, 2015).

The economy of Ethiopia is mainly dependent on agriculture that make it mostly vulnerable to the effect of zoonotic diseases (McDermott J, Grace S, Zinstaag., 2013) and majority of households have direct contact with domestic animals, creating an opportunity for infection and spread of disease. In the present study area all of the herds shared the communal grazing which allows unrestricted contact between animals that contributes the spread of brucellosis in extensive management system. The prevalence is linked to the practice of animal movement to communal watering points and other areas when searching for pasture and water (Abubakar *et al*., 2012).

Majority of the studies on cattle brucellosis have been carried out in central and northern Ethiopia which focused on dairy cattle's of urban and per-urban areas (Dinka and Chala, 2009; Megersa *et al*., 2011). However, the majority of livestock were found in rural areas where most households have direct contact with domestic animals and the habit of consuming raw milk, raw or undercooked meat is still a common practice, especially among rural communities (Kambarage *et al.*, 2003; Shirima *et al*., 2003). This could mainly be attributed to lack of knowledge of the zoonotic risks associated with the consumption of unpasteurized milk.

A number of reports have indicated the occurrence of livestock and human brucellosis is increasing (Dinka and Chala, 2009). However, it is difficult to note the general prevalence of animal and human brucellosis in the whole country due to lack of uniform studies in different parts of the country. Similarly, there were no studies undertaken on the seroprevalence,its associated risk factors and community awareness towards brucellosis. Accordingly, the study was undertaken to fill such gapswith the following objectives.

- To determine the overall seroprevalence of bovine brucellosis in Gambella and Itang district
- To assess potential risk factors for infection of bovine brucellosis in the study areas;
- To assess knowledge, attitudes and practices of owners about brucellosis in the study area.

2. LITERATURE REVIEW

2.1. Etiology

The genus Brucella belongs to the family Brucellaceae, order Rhizobiales, class Alpha proteo bacteria and phylum Proteo-bacteria. All proteo-bacteria are gram-negative, with an outer membrane mainly composed of lipopolysaccharides (Murray and Holt, 2005).Brucella species are facultative intracellular, gram-negative, cocco-bacilli, non-spore-forming, non-motile, non-sporulating, non-toxigenic, non-fermenting, that can infect many species of animals, including humans and non-capsulated(Mantur *et al.*, 2007).

Currently there are about ten species are recognized within the genus *Brucella* (Godfroid *et al*., 2011). The genus *Brucella* consists of six classic species that infect land animals namely; *B. melitensis, B. abortus, B.suis, B. ovis, B. neotomae* and *B. canis*. The *B. melitensis* biovars (bvs) 1-3, (mainly isolated from sheep and goats), *B. abortus* bvs 1-6 and 9 (from cattle and other bovidae), *B. suis*bvs 1-3 (from pigs), bvs 4 (from reindeer) and bvs 5 (from small rodents), *B. canis* (from dogs), *B. ovis* (from sheep) and *B. neotomae* (from desert wood rats). This classification is based mainly on differences in pathogenicity and host preference (Moreno *et al*., 2002). Brucella abortus is mainly infective for cattle, but occasionally other species of animals such as sheep, swine, dogs and horses may be infected. Although Brucella abortus infecting cattle has seven recognized biovars, the most reported of which are biovars 1, 2, 3, 4, and 9, with biovar 1 being the most prevalent. The distribution of biovars could be important in ascertaining the source of some infections (Neta *et al*., 2010). Cattle also become infected by *B. suis* and *B. melitensis* when they share pasture or facilities with infected pigs, goats, or sheep. The infections in cattle caused by heterologous species of Brucella are usually more transient than that caused by B. abortus (Bashitu *et al*., 2015).

2.2. Morphology

Brucellais Gram-negativecoccobacilli or short rods measuring from 0.6 to 1.5μm long and from 0.5 to 0.7μmwide, non-motile, non-spore forming, non-capsulated, non-flagellated, aerobic, facultative intracellular bacteria capable of invading, survive and

multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages (Gorvel, 2008).

The bacteria are usually arranged singly,and less frequently in pairs or small groups. The morphology of Brucella is fairly constant except in old cultures, where pleomorphic forms may be evident. The Brucella have no classic virulence genes encoding capsules, plasmids, pili or exotoxins and compared to other bacterial pathogen relatively little is known about the factors contributing to the persistence in the host and multiplication within phagocytic cells. Also, many aspects of interaction between Brucella and its host remain unclear (Seleem *et al.,* 2008; Sriranganathan *et al.,* 2010).

The Brucella is not truly acid-fast, but are resistant to decolonization by weak acids and thusstain red by the Stamp'smodification of the Ziehl-Neelsen's method. On suitable solid media,Brucella colonies can be visible after 2–3 days‟ incubation at 37^{o}C. After 4 day of incubation,Brucella colonies are round, 1–2 mm in diameter, with smooth margins. They are translucent and a pale honey color when plates are viewed in the daylight through a transparent medium. Whenviewed from above, colonies appear convex and pearly white. Later, colonies become larger andslightly darker (OIE, 2009).The cellular and colonial morphology of the Brucella species are similar in most respect. All*Brucella* species possess smooth lipopolysaccharide (SLPS) in their outer cell wall except *B. ovis*and *B. canis*, which have rough lipopoly-saccharide (RLPS) and protein antigens (Blasco *et al.,* 1994).

2.3. Epidemiology of Brucellosis

The epidemiology of cattle brucellosis is influenced by several factors including factors associated with disease transmission between herds, factors influencing the maintenance and spread of infection within herds (Crawford *et al.,* 1990). Understanding the epidemiology of brucellosis is therefore, vital for strategizing evidence based disease control measures. However, such information is inadequate in sub-Saharan Africa. Consequently, appropriate preventive measures have not been undertaken in this part of the world (McDermott and Arimi 2002).

2.3.1. World distribution

The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging. New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening (Pappas *et al*., 2006). The disease occurs worldwide, except countries which include Australia, Canada, Cyprus, Denmark, Finland, Netherlands, New Zealand, Norway, Sweden and the United Kingdom which has eradicated. This is defined as the absence of any reported cases for at least five years. However, the Mediterranean Countries of Europe, Africa, Near 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 (Robinson, 2003).

2.3.2. Global distribution of human brucellosis

Brucellosis is named after Sir David Bruce, who in 1886 isolated the causative agent from a soldier in Malta where the disease caused considerable morbidity and mortality among British military personnel. During the 19th century, brucellosis was thus known as Malta or Mediterranean fever (Buzgan *et al.,* 2010). Human brucellosis is also known by many different names such as intermittent typhoid, Rock fever of Gibraltar, and more commonly, undulant fever (Buzgan *et al*., 2010). Human brucellosis tends to occur more commonly in regions with less established animal disease control programs and in areas where public-health initiatives may be less effective. An estimated 500,000 new human *Brucella* cases were reported annually worldwide (Pappas *et al.,* 2006). Four species of *Brucella* have known pathogenicity for humans worldwide, these include; *B. melitensis*, *B. abortus*, *B. suis* and *B. canis* (Godfroid *et al*., 2011). However, *B. melitensis, B. arbotus*, and *B. suis* are highly pathogenic for humans with *B. melitensis* being the most pathogenic for humans (OIE, 2011).

Human brucellosis is known to be highly endemic in the Mediterranean basin, Middle East, Western Asia, Africa and South America (Pappas *et al*., 2006). Countries with the highest incidence of human brucellosis include Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman (Pappas *et al*., 2005). Syria had the highest annual brucellosis

incidence worldwide, reaching an alarming 1603 cases per million per year according to data from OIE (2004).In the United Arab Emirates, most cases are reported from Dubai, a popular international travel destination, underlining the importance of the disease in the field of travel medicine (Refai, 2002).

In the United States, brucellosis is much less common, with only 100-200 human cases reported each year. This decrease in cases in the United States is felt to be due to effective animal vaccination programs and milk pasteurization. In Europe, human brucellosis is thought to be associated with travellers and immigrants from the Middle East or the private import of dairy products from endemic areas (Georgi *et al.*, 2017). The World Bank (2011) ranked Dubai and Abu Dhabi as being the second and third, most popular medical tourism destination in the region behind Jordan (Refai, 2002).

2.3.3. Distribution in Africa

In Africa, bovine brucellosis was first recorded in Zimbabwe (1906), Kenya (1914) and in Orange Free State of South Africa in the year 1915 (Chukwu, 1985). However**,** still the epidemiology of the disease in livestock and humans as well as appropriate preventive measures are not well understood and such information is inadequate particularly in sub Saharan Africa. The importance of brucellosis reflects its widespread distribution and its impacts on multiple animal species, including cattle, sheep, goats, pigs and humans. While the importance of brucellosis is widely assumed, the benefits of programs to control it, relative to their costs, need to beassessing (Mc Dermot *et al*., 2002). Some countries in Africa where seroprevalence of brucellosis had been reported to be less than 10% were Benin 4.3%, Ethiopia 4.2%, and Ghana 6.6% (Kubuafor *et al*., 2000; Megersa *et al*., 2011).

According to the OIE (2009) bovine brucellosis is a reportable zoonosis and is of considerable socioeconomic concern. Most African countries are of poor socioeconomic status, with people living with and by their livestock, while health networks and surveillance and vaccination programs are virtually non-existent in most Africa (Mc Dermott and Arimi, 2002). In most low-income countries, there is much less public investment in veterinary and health services, with weaker surveillance and operational capacity. Such interventions are not feasible in many developing countries because of

poor surveillance programs, limited institutional capacity and lack of funds for livestock holder compensation (Zinsstag *et al*., 2007).

Table 1: Distribution of bovine brucellosis in some African countries

Country	**Host**	**No. tested**	**Prevalence (%)**	**Tests used**	**References**
Eretria	Cattle	15049	2.77	CFT	Scacchia *et al*., 2013
Zambia	Cattle	395	20.7	c-ELISA	Muma *et al*., 2013
Sudan	Cattle	250	2	ELISA	Senein and Abdelkadir 2012
Kenya	Cattle	393	1	c-ELISA	Kang‟ethe *et al*.,2007
Zimbabwe	Cattle	1291	5.5	c-ELISA	Matope *et al*., 2010
Somaliland	Cattle	153	1.96	RBPT	Ahmed, 2009
Nigeria	Cattle	220	5.45	RBPT	Bwala *et al*., 2015
Tanzania	Cattle	655	5.3	RBPT	Swai and Schoonman, 2010
Uganda	Cattle	423	5	c-ELISA	Makita *et al*., 2011
Gambia	Cattle	465	1.1	CFT	Unger *et al*., 2003
Senegal	Cattle	479	0.63	CFT	Unger *et al*., 2003
Ghana	Cattle	444	2.93	RBPT	Folitse, 2014
Cameroon	Cattle	840	9.64	i-ELISA	Shey –Njila, 2005

2.3.4. The Status of bovine brucellosis in Ethiopia

Even though, several serological surveys have showed bovine brucellosis is an endemic and widespread disease in Ethiopia, most of the studies on cattle brucellosis have been carried out in central and northern Ethiopia and do not provide an adequate epidemiological picture of the disease in different agro-ecological zones and livestock production systems of the country (Dinka and Chala, 2009; Megersa *et al.*, 2011).

The evidences of brucellosis in Ethiopian cattle have been serologically demonstrated by different authors. Most of the studies suggested a low seroprevalence (below 5%) in cattle under crop-livestock mixed farming (Berhe *et al*., 2007; Ibrahim *et al*., 2010; Adugna *et al.,* 2013). The evidences of *Brucella* infections in Ethiopian cattle have been serologically evaluated in different parts of the country by different authors in different production systemas indicated in (Table 2).

Table 2.Seroprevalence of bovine brucellosis in different parts of Ethiopia

Breed	Location	No. tested	Prevalence (%)	Tests used	References
Local	South east	180	1.4	RBPT	Donde, 2013
Local	West	1152	1	CFT	Adugna *et al*., 2013
Local	North	1968	4.9	CFT	Haileselassie *et al*., 2010
Mixed	Assela	304	14.14	RBPT	Deselgn & Gangwar, 2011
Mixed	Central	1238	2.9	CFT	Jergefa *et al*., 2009
Cross	Ambo	169	0	CFT	Bashitu *et al*., 2015
Cross	Derebrhan	246	0.2	CFT	Bashitu *et al*., 2015
Local	South east	862	1.4	CFT	Gumi *et al*., 2013
Mixed	Southern	811	1.66	CFT	Asmare *et al*., 2007
Local	EastShowa	1106	11.2	RBPT	Dinka and Chala, 2009
Local	Eastern	435	1.38	CFT	Degefu *et al*., 2011
Mixed	Wollega	406	1.97	CFT	Moti *et al*., 2012
Local	Arsi zone	370	0.05	CFT	Degefa *et al*., 2011
Mixed	Debrezeit	300	2	CFT	Alemu *et al*., 2014
Mixed	Alage	804	2.4	ELISA	Asgedom *et al*., 2016
Mixed	Asella	756	2.9	CFT	Tsegaye *et al*., 2016

Based on some reports, *Brucella* seroprevalence is higher in intensive farming system than within extensive cattle rearing systems. In Borena zone of Oromia region, the highest seroprevalence (50%) was documented using ELISA in Didituyura Ranch (Alem and Solomon, 2002). Tolosa *et al*. 2008 reported overall individual animal prevalence and herd prevalence of 0.77 and 2.9%, respectively in Jimma Zone. Reportsfrom North West, Tigray region (Haileselassie *et al*., 2010) and Southern Sidama zone (Asmare *et al*., 2010), recorded an overall prevalence of 1.2 and 1.66% following screening 848 and 1627 cattle from intensive and extensive system, respectively. Another study conducted on cattle brucellosis in traditional husbandry practice from 1623 cattle sera in southern and eastern Ethiopia showed that 3.5% of the animals and 26.1% of the herds were tested positive (Megersa *et al*., 2011).

2. 4. Possible Risk Factors for Infection

The prevalence of brucellosis is influenced by a number of risk factors related to productionsystems, biology of the individual host and environmental factors. These

include age, herd sizeand composition, hygienic status of the farm, rate of contact between infected and susceptibleanimals, farm bio-security and climate (McDermott and Arimi, 2002; Radostits *et al*., 2007).

2.4.1. Animal risk factors

Susceptibility of cattle to *B. abortus* infection is influenced by the age, sex and reproductive status of the individual animal. Sexually mature pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex. Susceptibility increases as stage of gestation increases (Tsegaye *et al.,* 2016). Most animals infected as adults remain infected for life. Herd size and animal density are directly related to prevalence of disease and difficulty in controlling infection in a population (Radostits *et al*., 2006).

2.4.2. Pathogen risk factors

Brucella abortus is a facultative intracellular organism capable of multiplication and survival within the host phagocytic cells. The organisms are phagocytized by poly morpho nuclear leucocytes in which some survive and multiply. The organism is able to survive with in macrophages because; it has the ability to survive phagolysosome. The bacterium possesses an unconventional non endotoxin lipopolysaccharide which confers resistance to antimicrobial attacks and modulates the host immune response. These properties make lipopolysaccharide an important virulence factor for survival and replication of *Brucella* (Ramirez *et al*., 2006).

2.4.3. Occupational risk factor

Risk factors for human brucellosis include the handling of infected animals, ingestion of contaminated animal products such as unpasteurized milk and milk products (including cow, goats and camel milk), meat and improper handling of cultures of *Brucella* species in laboratories. Laboratory workers handling *Brucella* cultures are at high risk of acquiring brucellosis through accidents, aerosolizing and/or inadequate laboratory procedures. In addition to this, abattoir workers, farmers and veterinarians are at high risk of acquiring the infection (Chain *et al*., 2005). In the rural parts of Ethiopia, for instance, human life is highly associated with livestock population in the different livestock production systems. In both pastoral and mixed livestock production systems people live

very closely with livestock having a high incidence of brucellosis and thus, are at higher risk of acquiring the infection (Gebretsadik *et al*., 2007).

2.4.4. Management risk factors

The spread of the disease from one herd to the other and from one area to another is almost always due to the movement of an infected animal from infected herd in to a non-infected susceptible herd (Addis, 2015; Tsegaye *et al.,* 2016). Large numbers of organisms are shed from the reproductive tract when infected cows abort. In cows which lactate following abortion, milk, including colostrum, is an important source of infection, and bacteria are excreted intermittently in milk throughout the lactation period. The fluid in hygromas caused by *Br. Abortus* infection may contain large numbers of organisms, but because of being restricted to the lesion they do not seem to be important in the spread of the disease (Tolosa, 2004).

2.5. Source of infection and mode of transmission

The most significant feature of bovine brucellosis epidemiology is the shedding of large numbers of organisms during the 10 days after abortion or calving of infected cows and the consequent contamination of the environment. The movement of infected cattle into a herd can result in transfer of the disease when cattle ingest the bacteria from aborted fetuses, placenta, and discharges from cows that have aborted or contaminated pasture or water (Park *et al*., 2005).

In cattle and other *Bovidae, Brucella* is usually transmitted from animal to animal by contact following an abortion. Infected animals after abortion or full-term parturition could be infectious for the other healthy animals. *B. abortus* may also be present in the milk, urine, semen, feces and hygroma fluids. Shedding in milk may be prolonged or lifelong, and can be intermittent. Many infected cattle can become chronic carriers and can shed lower numbers of organisms via milk and reproductive tract discharges, and also vertically transmit infection to subsequently born calves, and maintain disease transmission (McDermott and Arimi, 2002).

2.5.1 Transmission of brucellosis in animals

In cattle, transmission of *B. abortus* typically occurs through ingestion of live bacteria. It is transmitted among animals mainly through ingestion of contaminated feed and water and occasionally by inhalation of aerosols or by direct contact with infected materials (McDermott and Arimi, 2002; Maurin, 2005). Movement of infected cattle into a herd can result in transfer of the disease when cattle ingest the bacteria from aborted fetuses, placenta and discharges from cows that have aborted or contaminated pasture or water (Park *et al*., 2005). Venereal transmissions by infected breeding bulls to susceptible cows appear to be rare. Transmission may occur by artificial insemination when *Brucella* contaminated semen is deposited in the uterus but reportedly not in mid cervix (Cheville *et al.,* 1998). Venereal transmission is an important route of spread in pigs (Poester *et al.,* 2013).

2.5.2. Transmission of brucellosis in humans

Brucellosis in human also known as "undulant fever", "Mediterranean fever" or "Malta fever" is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. It affects people of all age groups but those less than 14 ages are less susceptible and of both sexes (Corbel, 2006).

The disease is mainly transmitted to humans through ingestion of contaminated animal products such as cheese and unpasteurized milk and by direct contact with infected animals through handling abortions, dystocia and parturitions (Shirima *et al.,* 2010). The source of naturally acquired brucellosis in humans is almost always from animal reservoirs, but very few cases of human to human transmission via blood transfusion, intrauterine infection, organ and tissue transplantation, sexual contact, and breastfeedinghave been reported (Godfroid *et al.,* 2011). The source of human infection resides always in domestic or wild animal reservoirs. The risk of contracting zoonosis from wildlife is higher in poor communities whose people and livestock interact with wildlife, commonly referred to as wildlife-livestock interface areas (Muma *et al*., 2014). Wildlife-livestock interfaces pose a challenge to human, animal and environmental health practitioners due to the complex and continuous cycle of disease transmission (Pandey *et al*., 2013).

From the public healthpoint of view, brucellosis is considered to be an occupational disease for people who work with infected animals, particularly farm workers, veterinarians, ranchers, game hunters and meat packaging factory employees (OIE, 2011). Human infection transmission typically occurs through three primary sources which include; consumption of unpasteurized dairy products where brucellosis is endemic, contact with infected livestock or wild animals, meat or tissues of animals and laboratory exposures. Infection may also occur by inhalation, conjunctival contamination, accidental ingestion, skin contamination especially via cuts and abrasion and accidental self-inoculation with *Brucella* S19 vaccine during field vaccination can lead to brucellosis transmission to handlers (WHO, 2006).

Brucella is highly infectious in laboratory settings and numerous laboratory workers who culture the organism have become infected. It is a frequently reported laboratory acquired infection (Singh *et al.,* 2015). *Brucella* organisms can be shed in the milk of infected animals for variable length of time, but for many, it can be shed for the life of the infected animal (Merck Veterinary Manual, 2012). Although *Brucella* agents can be transmitted directly and indirectly from its animal reservoir to humans, indirect transmission remains the highest overall risk and mainly occurs through the consumption of unpasteurized milk or dairy products (Godfroid *et al*., 2005). Fresh milk and dairy products prepared from unpasteurized milk such as soft cheeses and ice creams may contain high amounts of the bacteria and consumption of these is an important cause of human brucellosis (Makita *et al*., 2008).

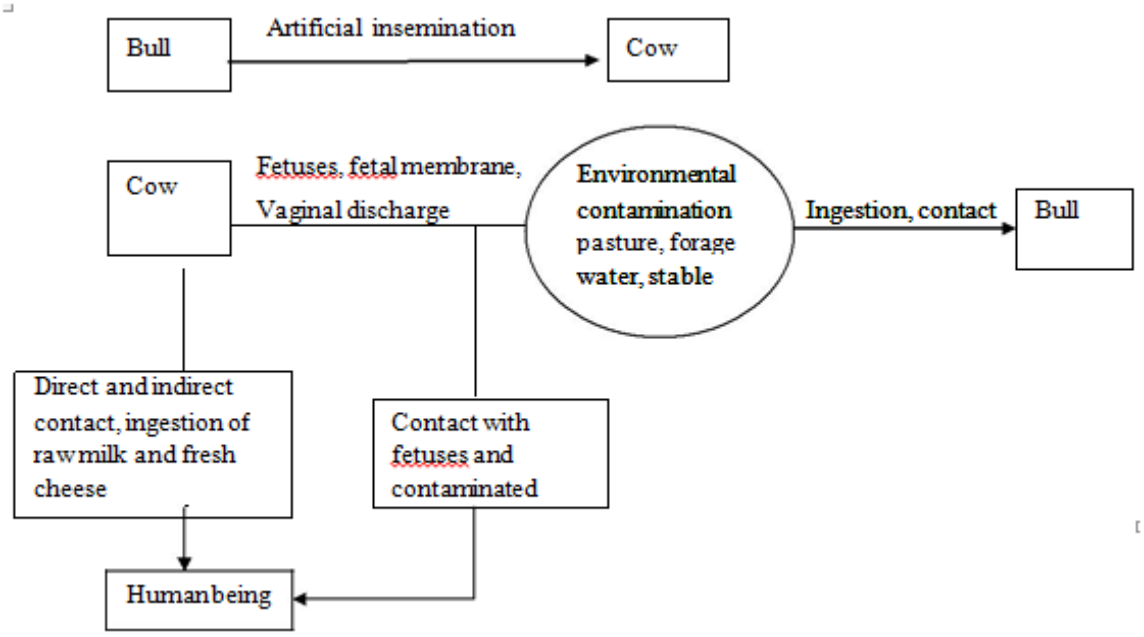

Figure 1: Mode of transmission of bovine brucellosis (*B. abortus*)

Source:Acha and Szyfres, 2001

2.5. Clinical Manifestation

Brucellosis could be suspected in any herd with history of abortion during the last stage of pregnancy (Poester *et al*., 2010). The major clinical sign in the first stage of the disease is abortion, but other signs due to localization of the organism may be observed. These signs include orchitis, epididymitis, hygroma, arthritis, metritis and subclinical mastitis among others (Radostits *et al*., 2007). However, numerous animals develop self-limiting infection or they may become asymptomatic latent carriers and potential execrators (WHO, 2003).

2.5.1. Clinical signs in animals

Females that are born into an infected area and get infected generally abort less than others. This explains the high level of abortions in newly infected herds and their relatively low frequency in herds where infection is enzootic. The udder is a very important predilection site for *Brucella* organisms. Infection in lactating, nonpregnant animal is likely to lead to colonization of the udder with excretion of *Brucella* organisms in the milk (Radostits *et al*., 2007). Retention of placenta and metritis are common sequels to abortion. Females usually abort only once, presumably due to acquired

defenses of the host chiefly macrophage and T-lymphocytes though specific antibody also plays apart (Radostits *et al*., 2007).

In contrast to other pathogenic bacteria,*Brucella* lack classical virulence factors, such as exotoxins, cytolysins, capsules, fimbria, plasmids, lysogenic phages, drug resistant forms, antigenic variation,but possibility that they might have unique and subtle mechanisms to penetrate host cells, elude host defenses, alter intracellular trafficking to avoid degradation and killing in lysosomes and modulate the intracellular environment to allow long-term intracellular survival and replication (Moreno and Moriyon, 2002; Delrue *et al*., 2004).

Brucella uses a number of mechanisms for avoiding or suppressing bactericidal responses inside macrophages. The smooth lipopolysaccharides that cover the bacterium and proteins involved in signaling, gene regulation, and trans-membrane transportation are among the factors suspected to be involved in the virulence of *Brucella* (Lapaque *et al*., 2005).When the bacteria prevail over the host's defenses, abacteremia is generally established. The bacteremia is always detected after 10 to 20 days and persists from 30 days to more than two months. If the animal is pregnant, bacteraemia often leads to the invasion of the uterus (Olsen & Tatum, 2010). At the same time, infection becomes established in various lymph nodes and organs, often in the udder and sometimes in the spleen (WHO, 2006).

2.7. Diagnosis

The advancement of a definitive diagnostic test for brucellosis remains an abstract target. The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis. In the history of microbiology, very few diseases have more diagnostic tests than brucellosis. Diagnostic tests are applied for the confirmatory diagnosis, screening or prevalence studies, certification, and surveillance in order to avoid the reintroduction of brucellosis (in countries where brucellosis is eradicated) through importation of infected animals or animal products (Godfroid *et al*., 2010).

Clinician must develop a high degree of clinical suspicion based on epidemiological information and history which are critical to making the clinical diagnosis. In all cases a

sample should be collected from the patient and laboratory testing should be requested as the definite diagnosis of brucellosis is impossible without laboratory confirmation (Bricker, 2002). In most developing countries, surveillance of zoonotic diseases is not recognized as a "one health" collaboration undertaking between veterinary medicine and human medicine. In addition, many countries lack diagnostic capacity and health infrastructure to diagnose the disease (Muma *et al*., 2014).

Despite the vigorous attempts for more than one century to come up with a definitive diagnostic technique for brucellosis, diagnosis still relies on the combination of several tests to avoid false negative and positive results (Poester *et al*., 2010). Several diagnostic methods have been used in the diagnosis of brucellosis, these includes; bacteriological detection methods, directly demonstration of antibodies using serological techniques and molecular methods (James, 2013).

2.7.1. Bacteriological method of diagnosis

Stained smears

A probable bacteriological diagnosis of *Brucella* can be made by means of the microscopic examination of smears from vaginal swabs, placentas or aborted foetuses, stained with the Stamp modification of the Ziehl-Neelsen staining method (Marin *et al*., 1996). However, morphologically-related micro-organisms, such as *Chlamydophila abortus, Chlamydia psittaci* and *Coxiella burnetti* can mislead the diagnosis because of their superficial similarity (Marin *et al*., 1996; Poiester *et al*., 2010). Accordingly, the isolation of *B. melitensis* on appropriate culture media such as Farrell's selective media is recommended for an accurate diagnosis (Farrell, 1974). Vaginal swabs and milk samples are the best samples to use in isolating *B. melitensis* from sheep and goats (Marin *et al*., 1996).

Cultural isolation

Definitive diagnosis of brucellosis is based on culture, serologic techniques or both. Isolation of the organism is considered the gold standard diagnostic method for brucellosis since it is specific and allows bio typing of the isolate, which is relevant under an epidemiological point of view (Bricker, 2002; Al Dahouk, 2003).

Isolation may be performed by culturing body tissues or secretions like blood, milk and virginal discharge (Poester *et al*., 2010). *Brucella* species can also be cultured from pus, joint and ascitic fluids. Vaginal swabs and milk samples are the best samples to use in isolating *Brucella* from animals (Roba, 2017). The identification of *Brucella* species in culture depends on a great deal of phenotypic traits such as: CO_2 requirement and biochemical tests (Bricker, 2002). Broth or agar can be prepared from powder media for culture of *Brucella* organisms. Due to the low *Brucella* load in the blood and milk, broth or a biphasic medium is recommended for improving sensitivity (Poester *et al*., 2010). However, for other specimens, solid media such as dextrose agar, tryptose agar, and trypticase soy agar, are recommended for primary isolation of *Brucella,* but some species, i.e., *B. ovis* and *B. canis* require addition of 5-10% of sterile bovine or equine serum to the culture media. Optimum pH for growth of *Brucella* varies from 6.6 to 7.4, and culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36-38°C. However, most strains grow between 20 and 40 ^{0}C (Poester *et al*., 2010).

The most widely used selective medium is the Farrell's medium (Marin *et al.,* 1996), which is prepared by the addition of six antibiotics to a basal medium to inhibit growth of contaminants that may prevent isolation of *Brucella* species. On suitable solid media, *Brucella* colonies can be visible after 2–3-days of incubation. After 4 days of incubation, *Brucella* colonies are round, 1–2 mm in diameter, with smooth margins. They are translucent and a pale honey color when plates are viewed in the daylight through a transparent medium (OIE, 2012).

In addition, fetal organs such as the lungs, bronchial lymph nodes, spleen and liver, as well as fetal gastric contents, milk, vaginal secretions and semen are samples of choice for isolation (Poester *et al*., 2006; Lage *et al*., 2008). Milk samples should be a pool from all four mammary glands. Non- pasteurized dairy products can also be sampled for isolation (Lage *et al*., 2008; Poester *et al*., 2010).

Inoculation into Guinea pig and mouse is another technique that has value for the isolation of *Brucella* when specimens are derived from potentially contaminated sources such as milk, cheese, semen, or genital discharges. Inoculation should be made

subcutaneously into Guinea pig or intravenously (0.1ml), or subcutaneously if the material is heavily contaminated, into mice. A guinea pig is killed 3 weeks post infection and 6 weeks after inoculation (Poester *et al*., 2010).

2.7.2. Serological diagnosis of brucellosis

Serological tests are relatively easy to perform and provide a practical advantage in detecting the prevalence of *Brucella* infection. The tests are crucial for laboratory diagnosis of brucellosis since most of control and eradication programs rely on these methods. Despite the development of numerous serological tests, no single test identifies all infected animals and a wide variation exists in estimates of their diagnostic accuracy (Abernethy *et al*., 2012; Adone and Pasquali, 2013). The serological tests are presumptive diagnosis for brucellosis in animals as well as human (OIE, 2012).

Several serological tests are used today, but most commonly used serological tests are screening tests (e.g., RBPT), monitoring or epidemiological surveillance tests (e.g., milk ring test), and complementary or confirmatory tests (complement fixation test, ELISAs). Selection of a given test should take into account the species of organism and the local regulations (Nielsen, 2002; Poester *et al*., 2010). Body fluids such as; serum, uterine discharge, vaginal mucus, and milk and semen plasma from suspected cattle may contain different quantities of antibodies of the IgM, IgG1, IgG2 and IgA types directed against *Brucella* (Zewdie, 2018).

Milk ring test

The milk ring test is based on agglutination of antibodies secreted into the milk. This test allows screening of large number of cattle by using milk samples from tanks or pools from several cows. This test is useful for monitoring cattle herds or areas free of brucellosis so it is classified as surveillance or monitoring test (OIE, 2009). Importantly, the number of false positive results is proportional to the number of cows secreting acidic milk due to colostrum or mastitis (OIE, 2009). A positive result indicates the presence of infected cattle in the herd so the test should be followed by individual serological test in the entire herd.

Rose Bengal plate test (RBPT)

This test was developed by Rose and Roekpe (1957) for the diagnosis of bovine brucellosis to differentiate specific *Brucella* agglutinins from non-specific factors. It is a rapid, slide-type agglutination assay performed with a stained *B. abortus* suspension at pH of 3.6-3.7 and plain serum. It does need special laboratory facilities and is easy to perform. It used to screen sera for *Brucella* antibodies.The test is an excellent screening test but may be oversensitive for diagnosis in individual animals, particularly vaccinated ones (Munoz *et al.,* 2005). Although the low PH (3.6) of the antigen enhances the specificity of the test, the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the test (Bricker, 2002).

Complement fixation test (CFT)

Complement fixation test (CFT) is another commonly used serological methods.Due to its high accuracy, complement fixation is used asconfirmatory test for *B. abortus*, *B. melitensis*, and *B. ovis* infectionsand it is the reference test recommended by the OIE for internationaltransit of animals (Gall *et al*., 2001; OIE, 2009). In most cases, the CFT is used on RBPT positive sera, but like the RBPT.The test hasdisadvantages such as high cost, complexity for execution, andrequirement for special equipment and trained laboratory personnel. Sensitivity of complement fixation ranges from 77.1 to 100% and its specificity from 65 to 100% (Gall *et al*., 2001; Perrett *et al*., 2010). The reagents include *B. abortus* CFT antigen, complement, amboceptor (haemolysin), ovine erythrocytes and test serum with Veronal buffer as the diluents (WHO, 2006; IBM, 2013).

Enzyme Linked Immuno Sorbent Assay

Enzyme Linked Immuno Sorbent Assay (ELISA) has become popular as a standard assay for the diagnosis of brucellosis serologically. It measures IgG, IgA and IgM antibodies and this allows a better interpretation of the clinical situation. The diagnosis of brucellosis is based on the detection of antibodies against the smooth LPS. Detection of IgG antibodies is more sensitive than detection of IgM antibodies for diagnosing cases of brucellosis but specificity is comparable (Araj, 2010; Sathyanarayan *et al*.,2011; Agasthya *et al*., 2012).

Compared to the conventional agglutination methods, ELISA is more sensitive in acute and chronic cases of brucellosis and it offers a significant diagnostic advantage in the diagnosis of brucellosis in endemic areas. This test is an excellent method for screening large populations for *Brucella* antibodies and for differentiation between acute and chronic phases of the disease (Gall *et al*., 2003). It is the test of choice for complicated, local or chronic cases particularly when other tests are negative while the case is under high clinical suspicion.

Fluorescence polarization assay (FPA)

It is based on the physical principle of the mass-dependent change of the molecules rotation speed in a liquid medium. The smaller the molecule, the faster it rotates and the depolarization of a polarized beam of light occurs. In FPA the serum sample is incubated with a specific *Brucella* antigen, conjugated with a fluorescent label. In case there are anti-*Brucella* antibodies in the serum, large fluorescently labeled antigen-antibody complex is formed, which can easily be distinguished from the unbound antigen negative control. FPA method has a high specificity but less sensitivity than I-ELISA (Mc Given *et al*., 2003). In Europe and the USA FPA method is used in programs to monitor and control the spread of brucellosis, but it requires special equipment and it is not suitable for rapid and easy testing.

2.7.3. Molecular methods

Molecular techniques are important tools for diagnosis and epidemiologic studies, providing relevant information for identification of species and biotypes of *Brucella* spp.,

allowing differentiation between virulent and vaccine strains (Le Flèche *et al*., 2006; López-Goñi *et al*., 2008). Moleculardetection of *Brucella* spp. can be done directly on clinical samples without previous isolation of the organism. In addition, these techniques can be used to complement results obtained from phenotypic tests (Bricker, 2002). Despite the high degree of DNA homology within the genus *Brucella*, several molecular methods, including PCR, have been developed that allow, to a certain extent, differentiation between *Brucella* species and some of their biovars (OIE, 2009).

Polymerase chain reaction

The polymerase chain reaction (PCR) is a recent and promising technique that allows accurate diagnosis of bovine brucellosis (Baddour, 2012). The technique is chosen based on the type of biological sample and the goal, i.e., diagnosis or molecular characterization or epidemiological survey. Most of the molecular diagnostic methods for brucellosis have sensitivity ranging from 50% to 100% and specificity between 60% and 98%. The DNA extraction protocol, type of clinical sample, and detection limits of each protocol, are factors that can influence the efficiency of the technique (Mitika *et al*., 2007).

2.8. Significance of the Disease

2.8.1. Economic significance

The assessment of the economic aspects of brucellosis, with emphasis on the low-income countries of Africa and Asia, is structured in three main parts. The first describes an overall framework for economic assessment of disease burdens and the impacts of potential control programs. The second part systematically reviews available animal, human and joint burden estimates from studies conducted in these regions. The third section provides estimates, when available, of different costs associated with brucellosis illness and its control. This section also comments on tools and approaches for assessing control programs that are of relevance to low and middle-income (Zamri-saad and Kamarudin, 2016).

Endemic brucellosis in low-income countries of sub-Saharan Africa and South Asia has multiple economic implications across agriculture and public health and broader socio-economic development sectors. Efforts to control the disease in low-income countries must take a different approach. Simply replicating past successes in brucellosis control

and eradication in high-income countries will not work. Low-income countries have at least a ten-fold higher burden of infectious disease from a wide variety of pathogens (Mc Dermott and Grace, 2013).

When brucellosis is detected in a herd, flock, region, or country, international veterinary regulations impose restrictions on animal movements and trade, which result in huge economic losses. The economic losses as well as its zoonotic importance are the reasons why programs to control or eradicate brucellosis in cattle (OIE, 2008).

In Ethiopia, information on losses specifically through brucellosis in the different types of production systems is sparse, except for Sintaro (1994) who reported an annual loss from brucellosis estimated to be 88,941.96 Ethiopian Birr ($5231 equivalent) among 193 cattle, largely due to reduced milk production and abortions (Chaffa State Farm, Wollo, from 1987 to 1993).

2.8. 2. Public health significance

Humans may become infected by ingestion of raw or unpasteurized dairy products, by direct transmission through contact with infected animals or by handling specimens containing *Brucella* species in laboratory. It also transmitted to human by direct contact with the skin or mucosa during parturition and abortion (Degefu *et al*., 2011; Ferede *et al.*, 2011; Addis, 2015).

Brucella abortus, B. melitensis and *B. suis*are highly pathogenic for humans (OIE, 2009). The majority of reported human brucellosis cases are caused by *B. melitensis, B. abortus,* and *B. suis*, in occurrence order, novel and atypical *Brucella* are also being investigated (Al Dahouk *et al*., 2013). Brucellosis remains the most common zoonotic disease in the world, with more than 500,000 new cases reported annually (Godfroid *et al.,* 2013); the actual number of cases, including undetected and unreported cases, is believed to be considerably higher (Al Dahouk *et al*., 2013).Brucellosis is often a neglected disease despite being endemic with high zoonotic potential in many countries (Poester *et al.,* 2013). The prevalence of human brucellosis differs between areas and has been reported to vary with standards of personal and environmental hygiene, animal husbandry

practices, and species of the causative agent and local methods of food processing (Chugh, 2008).

As compared to study of animal brucellosis, study of human brucellosis in Ethiopia is sparse with even less information on risk factors for human infection. For instance, out of 56 cases with fever of unknown origin, two (3.6%) were reported to be positive for *B. abortus* antibodies by RBPT and CFT (Jergafa *et al*., 2009). A study conducted in traditional pastoral communities by Ragassa *et al.* (2009) revealed that 34.1% patients with febrile illness from Borena, 29.4% patients from Hammer, and 3% patients from Metema areas were tested positive using *Brucella* IgM/IgG lateral flow assay. Studies conducted in high risk group such as farmers, veterinary professionals, meat inspectors and artificial insemination technicians in Amhara Regional State (Mussie, 2007), Sidama Zone of Southern People Nations and Nationalities Sate (Kassahun *et al.,* 2007), and Addis Ababa (Kassahun *et al*., 2006) found a seroprevalence of 5.30%, 3.78% and 4.8% by screening sera from 238, 38 and 336 individuals respectively. The discrepancy between and others might be due to difference in milk consumption habits and sensitivity of test methods used (Ferede *et al.,* 2011).

In South Sudan a fraught with several potential risk factors could fuel the dissemination of brucellosis to livestock and humans (Lado *et al*., 2012). The traditional pastoralist's practice of assembling several herds into cattle camps with close livestock-human interactions is one of the key milestones. Moreover, poor awareness is a risk milestone to occurrence and perpetuation of brucellosis in livestock which could create human health hazards (Yohannes, 2017). Further brucellosis risk indicators including the wide spread animal herder's practice of vulvar blowing, to facilitate milk let-down during cow milking (figure 4a) and the practice of direct udder-to-mouth consumption of raw milk (figure 4b) could exacerbate human brucellosis (Lado *et al*., 2012).

theidentification of infected herds and animals is of primeimportance (Aulakh *et al*. 2008). The treatment of brucellosis in the cow has generally been unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs (Radostits *et al*., 2000; Tolosa *et al*., 2004).

Prevention, control and eradication of brucellosis are a major challenge for public health programs. Although controlled or eradicated in animals in a number of developed countries through a combination of mass vaccination, test and slaughter programs, effective disease surveillance and animal movement control while the disease in humans has majorly been controlled through milk pasteurization (McDermott and Arimi, 2002; Pappas *et al*., 2006), re-introduction of brucellosis remains a constant threat, while in others, especially in the developing world, this disease continues to exert its devastating impact perpetuating poverty (Smits *et al*., 2004).

A very important approach to the control of brucellosis that is gaining more and more recognition in recent years is the One Health Approach to control and prevent human and animal brucellosis requires multidiscipline approach since neither veterinarian alone nor physician alone couldn't perform all approaches of control. So it requires participation of other discipline and farmers for effective control especially in developing countries where most people are living closer to animals (Pieracci *et al.*, 2016).

In the One Health framework veterinary, medical, environmental and allied professionals and experts collaborate together with the aim of identifying possible risk factors for this infection and design a suitable approach to combating the infection. Unfortunately, in many underdeveloped and developing countries, this kind of collaboration is non-existent or weak which gives room for brucellosis to thrive unchecked especially in rural populations (Beruktayit and Mersha, 2016).

In Ethiopia there have been national programs proposed for prevention and control of brucellosis through One Health Approach. However, at national and regional levels, no strategy is in place to control brucellosis. This is largely a result of lack of policy (Beruktayit and Mersha 2016). The successful prevention of this disease, which is so difficult in cattle production in the tropics, requires that, as far as possible, all available steps taken to combat it (Yohannes *et al.*, 2013).

Classification of endemic areas based on prevalence

Classification of endemic areas based on prevalence will enable initiation of appropriate control methods in endemic areas. Identification of low and high prevalence areas will greatly facilitate the implementation of appropriate control programs, and should ideally be combined with other strategies like accurate livestock census data and a livestock identification system (either simple ear notches or more sophisticated ear labeling system). In areas where the disease is less prevalent (livestock seroprevalence of less than 1%), cull policy with compensation may be recommended. For areas with high and moderate prevalence (>5%) under well-organized farming systems, we may recommend test and segregation policy by which animals with brucellosis will be isolated and products consumed after pasteurization (Yohannes *et al*., 2013).

Characterization of Brucella Species

Genotyping and identification of *Brucella* species based on molecular approaches have proved to be powerful tools to confirm the disease and to identify *Brucella* species and its *biovars* and *Brucella* like organisms. As a prerequisite, *Brucella* species identification should be undertaken to inform selection of the most appropriate vaccine (for example, *B melitensis* has recently been found infecting cattle in Kenya) and to enable differentiation of vaccine and wild-type strains (Muendo *et al*., 2012).

Vaccination

The WHO has long been involved in brucellosis surveillance and control, including research and development of vaccines to prevent animal brucellosis (Munir *et al*., 2010). Systematic vaccination of animals is recommended where the prevalence is greater than 5% (Holveic *et al.*, 2007). Vaccine increases individual resistance to systemic infection, and in infected animals decreases the probability of placental infection, abortion and massive shedding of infectious organisms (Ibrahim, 2010). In different parts of the world both live vaccines, such as *B. abortus* S-19, *B. melitensis* Rev-1, *B. suis* S-2, rough *B. melitensis* strain M111, and *B. abortus* strain RB-51 and killed vaccines, such as *B. abortus* 45/20 and *B melitensis* H-38 are available. Each vaccine has been reported to have its own advantages and disadvantages, with protection following localized

persistence of live vaccines preferred by most and showing efficacy in small ruminants and cattle (Thakur and Thapliyal, 2002).

Brucella abortus S19 Vaccine

The most widely used vaccine for the prevention of brucellosis in cattle is the *Brucella abortus* S19 vaccine, which remains the reference vaccine to which any other vaccines are compared. It is used as a live vaccine and is normally given to female calves aged between 3 and 6 months as a single subcutaneous dose of $5–8 \times 10^{10}$viable organisms. A reduced dose of organisms can be administered subcutaneously to adult cattle, but some animals may abort and excrete the vaccine strain in the milk. Alternatively, it can be administered to cattle of any age as either one or two doses of 5×10^{10}viable organisms, given by the conjunctival route; this produces protection without the risks of abortion and excretion in milk when vaccinating adult cattle (Seleem *et al.,* 2010). *Brucella abortus* S19 vaccine induces good immunity to moderate challenge by virulent organisms. Seed lots for S19 vaccine production should be regularly tested for residual virulence and immunogenicity in mice (Seleem *et al.,* 2010).

Brucella abortus strain RB51 vaccines

This is a recently developed vaccine and has replaced *Br. abortus* strain 19 in a number of countries as the approved calf hood vaccine because it does not interfere with serological evaluation (Asmare *et al*., 2010). *Brucella abortus* strain RB51 is a live stable rough mutant of *Br. Abortus*strain 2308, which lacks much of the lipopolysaccharide O-side chain and has been investigated as an alternative to strain 19 vaccines (Radostits *et al*., 2000). Adult vaccinations with *Br. abortus* strain RB51 only rarely causes abortion. One way to reduce the side effects of RB51 is to reduce the dose. When using the reduced dose of this vaccine (1×10^{10} colony-forming units [CFU]), on late pregnant cattle, no abortions or placentitis lesions are produced (Dinka and Chala, 2009).

Application of veterinary and human extension

The development of a national veterinary extension services in the country, is critical to promote awareness about brucellosis, its impact on livestock production and zoonotic risks. Everybody has responsibility to keep his environment, animals and own health care. Health education is another option to reduce occupational and food-borne risks. The ultimate prevention of human infection remains the elimination of infection among animals (Radostits *et al*., 2000). To lower your risk of getting brucellosis from natural source; avoid eating or drinking unpasteurized milk, cheese or ice cream and do not handle sick or dead animal bodies, but if you must, then use gloves and protective materials, cook meat thoroughly and disinfecting the area where the animals are aborted (Beruktayit *et al*., 2016).

3. MATERIAL AND METHODS

3.1. Description of the Study Area

The study was conducted in two purposively (logistic, accessibility) selected districts namely Gambella and Itang district of Gambella regional state from February 2019 to November 2019.

Gambella district is located in Agnuwa Zone,784 km far from Addis Ababa, surroundedby Kellem Wollega zone in the north, Itang district in the west, Ilubabor Zone in the east and Abobo district in the south. The administrative town of Gambella district, Abol is located 18 km to west from Gambella town, the capital city of the region and holds about 13 PAs. According to the National Meteorology Agency, Gambella Branch (2005), Elevations in Gambella District ranges from 400–600 meters above sea level; annual rainfall is 800-1600mm and temperature of the area ranges from 19.6 ^{0}C to 41.5 ^{0}C.Around 20% of the Woreda is covered by dense forest(CSA 2007).Mixed crop-livestock, production system practiced in the area. Cattle are used as assets and the source ofincome (GRAFDB, 2017).

Itang is the only special district in Gambella regional state, which is bordered on the south by Jikawo woreda, on the west by Lare woreda, on the north by Kellem Wollega zone and east by Gambellaworeda. The Administrative center of the district is Itang (Achewa) and it consists of about 26 PAs.It is located about 814 km, 48kmfrom Addis Ababa and Gambella town respectively. The districtlies between latitude and longitude of 8^{0}15'N, and 34^{0}35'E, respectively, annual rainfall is 900-1700mm and temperature of the area ranges from 19.6 ^{0}C to 42.5 ^{0}C. Extensive livestock production system is practiced in the area. Most types of livestock species are being reared. However, cattle are the predominant in the area. Cattle are used as assets and means of income generating beside the sources of food and play a vital role in order to have a wife in Nure culture.The district is also known in itsfish resource especially Nile-perch thatscoring upto150 kg.

Baro is the river which dividing Gambella town into two and which is used for transportation that across the country where also cross both districts and serve as the source of fish products in the area.There are about 95,760 heads of cattle kept in both

districts and the numbers of cattle found in each district are indicated in (Table 3)(GRAFDB, 2017).

Table 3:Cattle population of Gambella and Itang district.

District	**Cattle population**
Gambella	20,217
Itang	75,543
Total	95,760

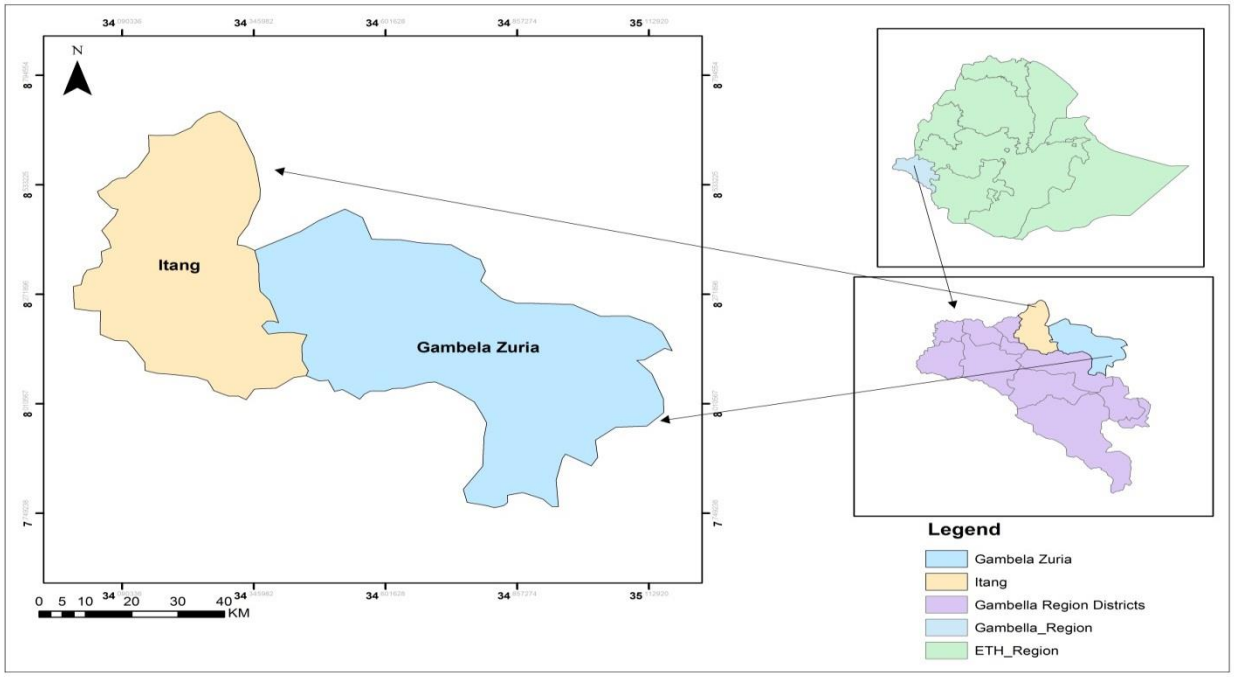

Figure 5 Map of the study area

3.2. Study animals

The local cattlebreedwith no history of vaccination against brucellosis inGambella and Itang districts were the study animals.Unrestricted animal movement, communal grazing

and watering, poor shelter, under feeding, etc., are livestock management problems, which might have their own part effect as factor for various animal diseases. Both sexes and different age group greater than six month were included in the study,while the cattle less than 6 months of age due to maternal antibody may interfere with test result.

3.3. Study Design

A cross-sectional epidemiological study was carried out to determine seroprevalence of brucellosis(at animal and herd level)andits association with different risk factors using two serological tests, Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) and questionnaire survey were used for KAP from February 2019 to November 2019.

3.4. Sampling Procedure and Sample Size Determination

The study districts were selected purposively on the basis of prior information on the problem, logistics, and accessibility. The selection of Peasant associations (PA's) was donebased on the proportions of PA's found in each districts. Accordingly, five PAs from Gambella district (Abol, Opagna, Bonga, Pinkwo and Ileyi), and eight PAs from Itang special district (Achewa, Baziel, Drong, War, Watgach, Mekod, Ibago and Eliya) were selected randomly. It was followed by made decision on the number of sampling herds (households) fromeachdistricts. Accordingly 20 and 60 herds were selected by systematic random sampling fromGambella and Itang special district respectively. The number herds taken from each PAs were based on the number of herds in the PAs. Therefore, (6,5,3,3,3) herds from (Bonga,Ileyi,Abol, Pinkwo, Opagna)PAs of Gambella district and Of 60 herds ofItang district about(10,10,10,9,8,6,4,3)herds from (Mekod,Watgach,Baziel,Achewa,Drong,War, Eliya, Ibago)PAs respectively were sampled randomly.The numbers of animals sampled from each PAs were also determined by the proportion of the cattle population existing in each PAs. Accordingly (30, 20, 14, 10 and 10) cattle from (Bonga, Ileyi, Abol, Pinkwo and Opagna) PAsand (60, 55, 45, 45, 40, 36, 20 and 15)cattle from (Mekod, Watgach, Baziel, Achewa, Drong, War, Eliya and Ibago) PAs respectively found in both districts were sampled by simple random sampling technique.Generally about 80 herds and 400 heads of cattle were sampled, of this about 77.5% (n=310) of the study animal were female and 24% (n=96) of them were young. The selection of PAs, herds and sampled animals were based on data obtained from the

districts agricultural office. Those cattle that housed in the same barns or under individual households were considered as one herd (Tolosa, 2004; Asgedom *et al.*, 2016).

According to data obtained from the district agricultural office, the number of households in each PA's varies from 80 to 150. Averages of 7 herds (households) were selected by systematic random sampling method from each PA. Animals above six months of age within the herds were selected using simple random sampling method. The Herd sizes were divided into three categories; small (≤15 heads of cattle), medium (≥15-30 heads of cattle) and large (≥30 heads of cattle) depending on number of animals (Boyazoglu, 1998). The number animals exists in each herds ranges from 15-200 (minimum and maximum) heads of cattle were found respectively.

To determine the desired sample size, there were no previous reports of bovine brucellosis prevalence in the present study area. Therefore, the average expected prevalence was assumed to be 50% for the area within 95% confidence interval (CI) at 5% desired precision as stated by Thrusfield (2007). Hence, using the formula, calculated sample for the current study becomes 384 heads of cattle; however, a total of 400 serum samples of both sexes were sampled in the study areas to increase the precision of the result.

$$n = \frac{Z^2 \times p_{expe} (1-p_{expe})}{d^2}$$

Where, n = required sample size

Pexp = expected prevalence

d =desired absolute precision

Z= confidence statistics

Table 4 The number of sampled animals from each district

District	Number of sampled animal
Gambella	84
Itang	316
Total	400

3.5. Sample and data collection

3.5.1. Blood Sample collection

Approximately 10 ml of blood was collected from the jugular vein of each selected animal using plain vacutainer tubes and needle. During the sampling, animals were restrained and the area was first disinfected by using 70% alcohol before puncturing. Identification of each animal was labeled on corresponding vacutainer tubes andcentrifuged at 2500/rpm for 5 minutes then after the serum were collected in to the sterile cryovial tube (2ml), to which animal's identification was coincided. Sera were kept at −20 °C in NAHDIC until serological tests were conducted. All serum samples were screened by Rose Bengal Plate Test (RBPT) at NAHDIC. The sera that tested positive to the RBPT were further subjected to the Complement Fixation Test (CFT) for confirmation at NAHDIC, Sebeta.

3.5.2. Questionnaire

A questionnaire was designed to collect information on factors that were believed to influence the spread and prevalence of *Brucella* infection. These include herd size (small <15 cattle; Medium 15-30 cattle; and large >30 cattle) and composition (bovine, caprine, ovine, canine),management system (extensive purchase source and replacement dairy cattle (own farm or outside source), handling of animal products (milk, meat) and handling of calving/abortion (parturition pen, burring, burning, thrown to env't). The following data were collected on animal attributes: sex, age of the animal (cattle: >0.6-3 years=young); 3-5years= adult; >5 years= old), and reproductive status, parity, history of abortion and retained fatal membrane and breeding (natural, AI).Questionnaire surveys with open and closed questions were used among the owners or attendants whose animals were tested. The data collected were ethical respected and confidential consideration

involvement, and the farmers interviewed from selected kebeles /districts were proportionally selected from each site by randomly sampling techniques.

3.6. Serological tests

3.6.1. Rose Bengal plate test (RBPT)

All serum samples collected were screened for Brucella antibodies using the Rose Bengal Plate Test (RBPT) at (NAHDIC) and the RBPT antigens were obtained from the National Animal Health diagnostic and Investigation Center (NAHDIC) Sebeta, Ethiopia .Testing was done according to the procedures stipulated by (OIE , 2009).Before performing test, antigen and sera are brought to room temperature. Then 30 µl of each serum sample was placed on a clean white tile and mixed with an equal volume of antigen. Subsequently, an equal volume of antigen was placed near each serum spot. The serum and antigen were mixed thoroughly using a clean tooth pick to produce a circle approximately 2 cm in diameter and the mixture was agitated gently for 4 min. at ambient temperature and the result was noted based on the presence or the absence of agglutination.

The interpretation was performed as follows: 0 = no agglutination, + = barely perceptible, ++ = fine agglutination, some clearing, +++ = coarse clumping, definite clearing. Those samples identified with no agglutination were recorded as negative and those with +, ++,+++ were recorded as positive.

3.6.2. Complement fixation test (CFT)

Complement fixation test (CFT) was used to all sera tested positive by Rose Bengal Plate Test (RBPT) for further confirmation.*B.abortus* antigen for CFT was used to detect the presence of anti-Brucella antibody in the sera like RBPT. Test was done according to the protocol of recommended by (OIE 2004) at NAHDIC, Sebeta. Antigen, control sera and complement were obtained from the BgVV, Berlin, Germany. The reading of results for the CFT was carried out as follows: When there was complete fixation (no hemolysis) with clear water supernatant, result was recorded as ++++, nearly complete fixation (75% clearing) as +++, partial hemolysis (50%) as ++ and some fixation (25% clearing) as +. Complete lack of fixation (complete hemolysis) was recorded as 0. For positive reactions final titrations was registered (OIE, 2004). Interpretation: Serum with strong reaction,

more than 75% fixation of complement (3+) at a dilution of 1: 5 and at least with 50% fixation of complement (2%) at a dilution of 1:10 and at dilution of 1:20 were classified as positive (OIE, 2004).

3.7. Data Analysis

All the data collected was entered in to Microsoft excel spread sheet and coded appropriately. Descriptive statistic was utilized to summarize data after coded and transferred to Statistical Package for the Social Science (SPSS) version 20. Two epidemiological parameters were generated namely individual animal and herd level seroprevalence. An animal was considered positive if it tested seropositive on both RBPT and CFT test. Individual animal seroprevalence was calculated by the number of positive animals divided by the total number of animals tested. Similarly, herd level prevalence was calculated by the number of positive herds with at least one seropositive animal in the herd divided by the total number of herds screened.

Univariable logistic regression analysis was used to select the individual explanatory variable that may predict the outcome variable in the model. The explanatory variables ($P \leq 0.25$) were further checked for multicollinearity using the variance inflation factor (VIF) and tolerance factor (TF) before multivariable logistic regression analysis. Variance inflation factor values of greater than 3 or tolerance less than 0.1 were considered the cut-off points for the collinearity diagnostics.The strength of association between outcome (*Brucella* seropositivity) and risk factors was assed using the odd ratio (OR). Multivariable logistic regression analysis was conducted to calculate the probability of disease happening as a function of several independent variables. The backward elimination procedure was used to eliminate the factors that were not significant at $P<0.05$ in overall model. Factors that were significant ($P \leq 0.05$) were retained in the final model and model fit was observed using the Hosmer-Lemeshow test.

4. RESULTS

4.1. Seroprevalence of Bovine Brucellosis

From the total of 400 Animals, 90(22.5%) male and 310(77.5%) female animals above 6 month of age were sampled and tested for B. antibodies. Of which 19 (4.75%) (95% CI

1.04-8.05) were positive to RBPT and positive sera were further retested by using CFT and the combined result (RBPT and CFT tests) 8 (2%) (95% CI: 0.75-3.2) sera were confirmed seropositive which giving over all seroprevalence of 2% (Table 5). Out of 80 herds included in the study, 2 herds from Gambella and 4 herds from Itang or 6 (7.5%) were found seropositive using RBPT+CFT with at least one seropositive animal in the herd. The individual animal seroprevalence of bovine brucellosis in the two district of Gambella region ranged from 1.89% to 2.38% (Table 5). Comparatively, higher seroprevalence of brucellosis was recorded in Gambella District (2.38%) than Itang District (1.89%).

Table 5 Overall individual animal and herd level brucellosis seroprevalence

Individual animal level prevalence				Herd level prevalence		
District	**NA**	**RBPT +**	**RBPT+CFT**	**NH**	**RBPT+**	**RBT+CFT**
Gambella	84	6(7.14%)	2(2.38%)	20	4(20%)	2(10%)
Itang	316	13(4.11%)	6(1.89%)	60	15(25%)	4(6.7%)
Total	400	19(4.75%)	8(2%)	80	19(23.75%)	6(7.5%)

NA=number of tested animals, NH=number of tested herds

4.2. Risk factors analysis

4.2.1. Animal level risk factors analysis

The result of Univariable analysis had shown the association of predictor variable and *Brucella* seropositivity (Table 6). Accordingly, seroprevalence of bovine brucellosis was not significantly related with study districts (P>0.05). Even though there were no significant difference among study districts and *Brucella* seropositivity, slightly higher proportion of seropositivity was observed in Gambella district (2.38%) when compared to Itang district (1.89%). Sex had no a significant associationwith brucellosis seropositivity (P>0.05) despite females having a slightly higher proportion of infection 2.25% (n=310) compared to males 1.1% (n=90). Seroprevalence of bovine brucellosis was significantly related with cows had history of RFM (P<0.05) and aborting cow (P<0.05). Age was also found a significant factor for brucellosis infection (P< 0.05) with old age having a higher

proportion of infection. Of 310 female animals tested 42 (13.5%) showed history of abortion and was significantly associated with seropositivity (P< 0.05), 43 (13.9%) with history of retained placenta, 84(27.1%) were pregnant, 60(19.3%) were lactating and 81(26.1%) dry, heifer and calves).

Table 6 Univariable logistic regression analysis of common risk factors associated with Brucella seropositivity at individual animal level

Factor	N. tested	CFT+ (%)	OR	95%CI	P-value
Districts					
Gambella	84	2(2.3%)			
Itang	316	6(1.9%)	0.794	(0.157-4.005)	0.779
Sex					
male	90	1(1.1%)			
Female	310	7(2.25%)	2.056	(0.250-16.935)	0.503
Age					
young	96				
adult	143	2(1.4%)	4.25	(2.75- 26.35)	0.051
old	161	6(3.7%)	7.861	(1.098-53.726)	0.040
Historyof abortion					
no	268	4(1.5%)			
yes	42	3(7.1%)	69.22	(8.25-78.51)	0.001
Historyof RFM					
no	267	2(0.7%)			
yes	43	5(11.6%)	28.784	(5.60-147.75)	0.000
RP-status					
Lactating	60				
Dry/heifer	166	2(1.2%)	35.989	(0.317-56.69)	0.896
Pregnant	84	5(5.9%)	0.208	(0.039-1.098)	0.064

N=number of tested animal OR= Odds Ratio, CI = Confidence Interval, RP=retain placenta

4.2.2. Herd level risk factors analysis

The herd level Univariable logistic regression analysis revealed that herd sizes were found to be strongly associated with seropositivity to *Brucella* infection (P < 0.05). There was no significant difference of *Brucella* seropositivity according to district difference (P>0.05). However relatively higher proportion of seropositivity was observed in Gambella District (10%) when compared to Itang District (6.7%).The study also fails to detect a significant variation in *Brucella* seropositivity among other risk factors at herd level (Table 7).

Table 7 Univariable logistic regression analysis of common risk factors associated with Brucella seropositivity at herd level

Factors	Categories	NH	CFT +ve	OR	95%CI	P-value
District	Gambella	20	2(10%)			
	Itang	60	4(6.7%)	0.999	(0.964-1.036)	0.971
	Small*	27				
Herd size	Medium	25	1(4%)	0.037	(0.011-0.993)	0.042
	Large	28	5(17.8%)	0.072	(0.013-0.881)	0.038
New. animal	No*	57	2(3.50%)			
	Yes	23	4(17.4%)	4.636	(0.79-27.25)	0.089
Maternity pen	No*	67	4(5.9%)			
	Yes	13	2(15.4%)	1.017	(0.109-9.497)	0.981
Disposal after birth	No*	71	5(7.04%)			
	Yes	9	1(11.1%)	1.028	0.194-5.431	0.974

NH=number of herds, *= reference, OR = Odds Ratio, CI = Confidence Interval

The result of multivariable logistic regression analysis showed important risk factors for *Brucella* seropositivity (Table 8). Risk factors with p-value ≤0.25 in the univariate logistic regression model were included in the separate multivariable logistic regression model fitted. Accordingly, Age, Herd size,history of maternal abortion, introduction of new animal, reproductive status (pregnancy) and history of retain fetal membrane were significantly associated with *Brucella* seropositivity were included in the final logistic regression model.Of all of this, in the final analysis though animal's seropositivity was significantly influenced more by herd size, maternal abortion and prior history of retain fetal membrane, while introduction of new animalwas not included in the multivariable regression because of its multicollinearity with herd size. Age and reproductive status(pregnancy) were found not significantly associated with Brucella infection, and the rest of the variables were not included in the final model. Thus multivariable logistic regression analysis showed that animals involved in the large herd are 9.4 times more likely to be at higher risk for *Brucella* infection than animals in small herd with (95% CI: 1.092-82.483, OR=9.4 P<0.05).Similarly, the multivariable regression analysis revealed that the seroprevalence of brucellosis was significantly associated with animal which had prior history of retain fetal membrane and those animal with RFM were found to be32 times more likely to be at higher risk for *Brucella* infection compared with no history of

RFM with (95% CI: 3.781-273.8, OR=32.1, P<0.05). Seroprevalence of brucellosis was also significantly associatedwith female animals those had prier history of abortion (95% CI: 5.759-12.389, OR=7.8, P=0.003).This might be explained by the fact that abortion is typical outcomes of brucellosis.

Table 8 Multivariable logistic regression analyses identifying the association of potential risk factors to Brucella seropositivity in cattle

Factors	Categories	OR	95% CI	P-value
Herd size	Small (<15 heads of cattle)ref*			
	Medium(>15-30 heads of cattle)	0.257	(0.049-1.353)	0.052
	Large (>30 heads of cattle)	9.481	(1.092-82.483)	0.040
HRM	No*			
	Yes	32.182	(3.781-273.8)	0.001
HMA	No*			
	Yes	7.8	(5.759-12.389)	0.003

OR= Odds ratio, CI= confidence interval,*=reference category, HMA=history maternal abortion, RFM= history of retain fetal membrane.

4.3. Questionnaire Survey

4.3.1. Socio-demographic characteristics of respondents

From the total of 80 respondents selected systematical, about 20(25%) and 60(75%) of them were from Gambella and Itang district respectively, and 2 and 4 totally (6) of their herds were found seropositive to Brucella infection respectively. Of the total households interviewed, 76.25% of them were illiterate, while 18.75 % of them were able to write and read, only 5% of them wereattended 6-8 grade education and none of them were proceeded this level. Majority of the respondents(83.75%) were male and 16.25% female, and found with 4 and 2 of their herds were positive respectively(Table 9).

Table 9 Socio-demographic characteristics of respondents in relation to herd seropositivity according to District

Variables	Categories	NR	NPH(CFT)
District	Gambella	20(25%)	2(33.3%)
	Itang	60(75%)	4(66.7%)
Educational Status	Illiterate	61 (76.25%)	5(83.3%)

	Write and read	15 (18.75%)	1(16.7%)
	6-8 grade	4(5%)	
Sex of respondents	Male	67 (83.75%)	4(66.7%)
	Female	13 (16.25%)	2(33.3%)

NR= number of respondents, NPH=number of positive herds

4.3.2. Herd management and husbandry systems of respondents

From the total households interviewed, 88.75% of the respondents were gained the skill from their parents and found with 5 positive herds, only 11.25% of them were acquired skill from extension/agricultural training, and found with 1 seropositive herd. Regarding the housing type, 90% of the herds were housed in corral and about 10% were housed in barn/open field and holds 4 and 2 positive herds respectively. Only 16.25% farmers were had separating maternity pen and found with 1 seropositive herd, most of the respondents (83.75%) had no maternity pen and 5 seropositive herds were with them (Table 10).

Table 10 Response of respondents on herd management and husbandry system

Variables	Categories	NR	NPH
Source of skill	Agri,training/Extension	9(11.25%)	1(16.7%)
	Parent	71(88.75%)	5(83.3%)
Housing type	Barn/Open field	8(10%)	2(33.3%)
	Corral	72(90%)	4(66.7%)
Separation of maternity pen	Yes	13(16.25%)	1(16.7%)
	No	67(83.75%)	5(83.3%)

NR= number of respondents, NPH=number of positive herds

4.3.3. Knowledge-attitudes and practices of farm owners about brucellosis

The majority of herd owners or respondents (87.5%) was not aware of bovine brucellosis and holds all positive herds. Respondents were also interviewed to describe the occurrence of some reproductive problems that causes abortion and Most of the respondents (86.25%) had no knowledge on causes of abortion and as brucellosis cause abortion in cattle, and found with most of (5) positive herd. The practices of disposing after birth were done mostly (96.25%) in the way thrown to the environment, with shared 100% of positive herds. About 95% of respondent were not separating aborted animal and found with all positive herds. The majority of the respondents consume raw milk (93.75%) and about 5 of their herds were positive. Similarly, most of the farmers (81.755%) have habit of assisting cows during parturition, without using of protective glove; they shared 4 positive herds of all positive herds (Table 11).

Table 11 Knowledge-attitudes and practices of farm owners about brucellosis

Variables	Categories	NR	NPH
Awareness about brucellosis	Yes	10 (12.5%)	
	No	70(87.5%)	6(100%)
Awareness about Abortion	Yes	11(13.75%)	1(16.7%)
	No	69(86.25%)	5(83.3%)
Separation of aborted cow	Yes	4(5%)	
	No	76(95%)	6(100%)
Proper disposal of after birth	Burial/burning	3(3.75%)	
	Thrown	77(96.25%)	6(100%)
Raw milk consumption	Yes	75(93.75%)	5(83.3)
	No	5(6.25%)	1(16.6%)
Assisting cow during parturition	Yes	65(81.25%)	4(66.7%)
with out glove	No	15(18.75%)	2(33.3%)

NR=number of respondent, NPH=number of positive herds

5. DISCUSSION

The cross-sectional serological study, attempted to look the status of bovine brucellosis in two districts of Gambella regional state, south western Ethiopia. The study revealed that, the overall seroprevalence of *Brucella* antibodies determined RBPTcombined withCFT was low prevalence (2%). This finding was slightly in agreement with other studies conducted by different authors on cattle under similar production systems in different parts of Ethiopia; 1.7% from Arsi Zone (Tsegaye *et al*., 2016), 1.97% from East Wollega (Moti *et al*., 2012), and 2% from Sudan (Senein and Abdelgadir, 2012) abroad the country.However, higher prevalence was observed by various other authors than the present study in other parts of the country (Hailemelekot, 2005, Kebede *et al.*, 2008,Hailessilasise et al., 2010, Deselgn and Gangwar, 2011, Asgedom *et al*.,2016).4.63% 11.1%, 7.7%, 14.14%, 3.3%seroprevalence was recorded respectively.

On the other hand the lower prevalence than the present study was reported by different authors;Tefera (2006) with prevalence of 1.13% in intensive and extensive farms of Addis Ababa and Sululta, Berhe *et al. (*2007) who found an overall prevalence of 1.49% in extensive and semi-intensive farms ofTigrayRegion,Degefu *et al*. (2011) who found an overall prevalence of 1.38% from Agro pastoral cattle's of Jijjiga,Yohannes (2017) withprevalence of 1.3 in Humbo districts of Wolaita zone, Roba (2017) with prevalence of 1.1% in Dida Tuyura Ranch and pastoral herds of Borena zone.

The differences in prevalence observed between the reports from different parts of Ethiopia and the present study may be due to differences in herd size, sample size, agro ecological, management conditions and the presence or absence of infectious foci, such as *Brucella*-infected herds, which could spread the disease among contact herds.

The present study showed that there was non-significant difference in seroprevalence of brucellosis among two study districts (Gambella and Itang). This could be due to similarity in management system andagro ecological.

In the present study, the seroprevalence of bovine brucellosis was not statistically significant between the sexes; though the result showed that infection was higher in female (2.25%) than male (1.1%). This finding was in agreement with the findings of Hailemelekot *et al.* (2007) in Tigray region, Berhe *et al. (*2007) in Tigray region,

Deselegn andGangwar (2011) in Asella dairy farm, Asgedom *et al.*The lower prevalence of male reactors in this study could be due to smaller number of males tested as compared to female and it has also been reported that the organism prefer gravid uterus for growth and multiplication relative to testicle and epididymis (Megersa *et al*., 2011). (2016) in and around Alage districts who reported higher prevalence in female than male. Although nocontrolled study has been conducted on the relative susceptibility of female and male cattle to brucellosis, based on reactor rates it is probable that bulls are more resistant than sexually mature heifers and cows, however, are less resistant than sexually immature heifers (Nicoletti, 1980).The lower prevalence of male reactors in this study could be due to smaller number of males tested as compared to female.

This study revealed that, all infected animals were adult though there was not statistically significant difference (P>0.05) in seroprevalence of *Brucella* among different age groups. This finding was in agreement with Lidia (2008) in central highland of Ethiopia and Nuraddis *et al,*), (2010) in selected site of Jimma zone, who reported only older age category reactors,Megersa *et al.* (2011), Tsegay *et al.* (2015), Asgedom *et al.* (2016). According to some authors (Bekele *et al*., 2000, Roba, 2017, Yohannes, 2017) susceptibility to brucellosis is reported to increase as the animals approach to the breeding age. Thus, sexually mature cattle are more susceptible to infection with *Brucella* organism than sexually immature animal of either sex (Taye, 2005). In this study there was no seropositive reactor in animals less than 3 years of age; This finding was in agreement with the prevalence report of 0.0% in nullparous animals by (Berhe *et al*., 2007), (Kebede *et al*., 2008)(Ibrahim *et al*., 2010). This shows that brucellosis is highly related with age and sexual maturity of animals.

In this study herd size remained significantly associated with Seropositivity to brucellosis. This finding was in agreement with the reports (Asmare *et al*., 2010; Hailesillasie *et al*., 2010; Ibrahim *et al*., 2010; Adugna *et al.*, 2013; Yohannes, 2017). An increase in herd size is usually accompanied by increase in stocking density, as well as an increase in risk of exposure to infection. Stocking density is an important determinant of the potential for transmission between susceptible and infected animals (Omer *et al.*, 2000). There is also undeniable fact that the spread of the disease from one herd to another herd and from one area to another is almost frequently due to the movement of an infected animal from an infected herd to a non-infected susceptible herd (Radostits *et*

al.,2000). Therefore, brucellosis should never be viewed as the disease of individual animals, but should be considered in the context of herd and also the animal population in the region.

The cow with history of retain fetal membrane was significantly associated with seropositivity in the present study (p=0.001).Seropositivity to Brucellosis was higher inanimals with history of retain fetal membrane(11.6%) compared to with no history of RFM (0.75%) animals.Association between brucellosis seroprevalence and occurrence of RFM also reported (Berhe *et al.*, 2007; Ibrahim *et al*., 2010; Adugna *et al.*, 2013; Tsegaye *et al*., 2016; Yohannes, H., 2017).

Even though pregnancy not significantly associated with seropositivity, pregnant cattle were showed more susceptible (5.9%) than nonpregnant (2.4%) to *Brucella* organism. This finding was in agreement with the reports of (Omer *et al*., 2000; Adugna *et al.*, 2013; Yohannes, 2017) in their study found that pregnancy status of cattle has no significant effect on the seroprevalence of brucellosis.

This study revealed that, the history of previous abortion was foundsignificantly associated with Seropositivity to brucellosis with (P=0.003). Among the cows that hadhistory of previous abortion was exhibited more than 7% (3/42) Brucella antibody in their serum than those cows which had no previous history of abortion1.5% (4/268). This was in agreement with other authors (Berhe *et al.*, 2007; Ibrahim *et al*., 2010;Adugna *et al.*, 2013; Tsegaye *et al*., 2016; Yohannes, 2017).

The information gathered with questionnaire survey has provided about the socio-demographic characteristics of the respondents, herd management and husbandry practice, knowledge- attitude and practices of cattle owners about brucellosis in selected districts of Gambella region. The educational status attained by majority of the respondents was low which falls between illiterate and lower grades.This low level of knowledge may lead to be at higher risk of acquiring and transmission of the disease, reduced production gained from animals because of the effects of the disease.Knowledge of diseases is a crucial step in the development of prevention and control measures

(Gessese *et al*., 2014). Irrespective toenormous efforts of the government institutions to improve animal production in the areas, most farmers were not familiarized with new technologies.In addition to this, proper disposal of aborted materials, unprotected contacted with infected tissues(feotus, retain placenta), the habit ofraw milk consumption, use of a separate parturition pen and assisting parturition by using protective gloves were not under consideration. Generally, the awareness of the respondents was very low.These could have effect on the transmission of the disease within and between the herds and human.This finding was in agreement with previous studies in extensive livestock production system (Ragassa *et al*. 2009; Megersa *et al.* 2011; Adugna *et al*., 2013). The occurrence of brucellosis in humans is associated with contact with domestic animals, exposure to aborted animals and assisting animal parturition (Kozukeev *et al.* 2006). In this study, the majority of the respondents have the habit of drinking raw milk and assisting parturition without using protective glove. This concludes that the lack of awareness about the impacts of the disease and this in turn, contributes to the spread and transmission of the infection to human in the area.Thus, there is a need to design and implement control measures aiming at preventing further spread of the disease in the Region through the use of better management practices.

6. CONCLUSION AND RECOMMENDATIONS

The present study showed thatthe seroprevalence of bovine brucellosis was found to be low inGambella and Itang special districts of Gambella region. The finding of positive serological reactors indicates the presence of foci of infection that could serve as sources of infection for the spread of the disease into unaffected animals and herds. The study revealed that herd size, abortion and retain fetal membrane were found to be significantly associated with *Brucella* seropositivity. The study also clearly showed that cattle owners had less knowledge of the disease and are at higher risk of acquiring the infection that was realized by consuming raw milk, assisting parturition and handling of aborted materials without using protective gloves. Based on the above conclusions, the following recommendations wereforwarded.

- Controlling of unrestricted animal movement need to be practiced in the area.
- Awareness creation should be carried out targeting Brucellosis for livestock owners as well as general public in order to avoid direct or in direct contact with infected animals and their products.
- Boiling of milk before consumption and isolation of calving animalsshould be carried out.
- Deep burring aborted fetuses and fetal membrane should be practiced.
- In addition to this the freely movement of the animal across the country were observed during sample collection that may contributed in the disease occurrence, transmission and inducing fear in trans-boundary diseases, Hence calling for due attention in the establishment of quarantine station in the area.

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8. ANNEXES

Annex 1: Data recording format for blood sampling

Epidemiological investigation of Brucellosis in cattle of study area

Region_____District _____PA/Town _____village_____

S/No	Owner name	District	PA	Herd Size	Animal origin	Age	Sex	Breed	Parity	Mating System	RP status	History of	Frequency of abortion	Gestational stage of abortion	History of RP, Still birth	Lab. result (+/-)	
																RBPT	CFT
1																	
2																	
3																	
4																	
5																	

PA= Peasant Association; RP= Retained placenta (Yes/No); History of maternal abortion (Y/N); RP= StatusReproductive Status (Pregnant, Lactating, Dry cow and Heifer); Mating System (natural or AI); Origin of the animals (born/bought).

Annex 2: Questionnaire survey for the assessment of brucellosis and associated risk factors.

Interview intended for livestock owners / respondents in the Study area

I. General Information on demographic characteristics of the respondents

Name of respondent _____ Sex_____Zone_____

District _____PA/Town_____Mob.No_____

1. Education level? a. illiterate b. read and write c. 6-8 grade d. above 12

2. How did you acquire skills to raise cattle/farming?

a) Agricultural training (level) b) From extension agents

c) From parents d) other

II. Information on herd (husbandry and management system)

1. Herd type and size in your farm

Type of cattle	Number of animals
Lactating cows	
Pregnant cows	
Dry cows	
Heifers	
Bull / ox	
Calves	

2. What is the feeding management of cattle?

a. Communal and free grazing b. Private and free grazing

c. Tether d. Stall feed

3. How are the average parity status and the calving interval of the dairy cows in your farm?

Cow identification (cross/local)	Parity status	Calving interval

4. How is the housing management/type?

a) Barn (Separately or mixed with other livestock)

b) Corral (Separately or mixed with other livestock)

c) Open field (Separately or mixed with other livestock)

d) Within the family house (Separately or mixed with other livestock)

e) Others

5. What are your culling criteria?

a) Disease b) old age c) infertility d) poor production e) other

6. Where do you get your replacement stock?

……

7. General farm hygiene / cleanliness

a) Very good b) Good c) Satisfactory d) Poor

III. Knowledge-attitudes and practices of farm owners about brucellosis

1. Have you ever seen reproductive problem in your farm? a. Yes b. No

2. Are you aware of any disease that causes abortion? a. Yes b. No

If yes, what is the local name for disease that causes abortion?

……

3. Do you know about brucellosis? a. Yes b. No

4. Do you think brucellosis is a zoonotic disease? a. Yes b. No

5. If yes through which means disease can transmit?

……

6. Was there any occurrence of abortion / still birth in your farm? Y/N

7. If your answer is yes, in which of the cows and at which time of pregnancy?

Cow identification Time of abortion

Heifer

Cow at first calving

Cow at second

Cow at third calving and more

8. How many abortions/still births or retained after birth have you encountered during the last three years? a. Number of abortion---------- b. Number of still birth----------
c. Number of retained fetal membrane----------

9. Do you separate cows during parturition? a. Yes b. No

10. Do you separate aborted animal from other? a. Yes b. No

11. Do you dispose after birth? a. Yes b. No

12. If yes how do you dispose of the after birth?

a. Burning b. Burying c. Both d. Thrown to the environment (open dump)

13. Is there frequent contact between your herds and other animals? a. Yes b. No

14. Did you see any testicular swelling? a. Yes b. No

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

16. Do you boil milk? a. Yes b. No

17. Do you consume raw meat of cattle? a. Yes b. No

18. Do you assist cow during parturition? a. Yes b. No

19. If your answer is yes, do you use protective glove during assisting? a. Yes b. No

20. What do you do with the known *Brucella* infected animals?

a. Separate the infected animal b. Sell to neighbor

c. Sell to market d. take to the local veterinarian clinic

……

21. Have you introduced new animals into your herd in the last one year? a. Yes b. No If yes, how many Cattle?

22. Did the herd been tested for brucellosis?

……....
.

23. Did vaccinations for brucellosis been carried? Y/N, when? ……

Thank you very much for participation and cooperation in this study!!!

The outcomes of the study will be shared among stakeholders whenever available for the purpose of improving animal, public and environmental health. Do you have any comment or question about the interview and our conversation?

Annex 3: Rose Bengal Plate Test (RBPT) reagents, material and equipment and procedure (OIE, 2016).

Reagents and materials required for RBT

Reagents:

1. Rose Bengal Test *Brucella* antigen
2. Positive control sera (from previously positive serum)
3. Negative control sera (from previously negative serum)
4. Test sera

Materials

1. Plate
2. Micro pipette of 30 µl
3. Micro pipette tips
4. Applicator
5. Tube of serum collection
6. Magnifying glass
7. Vacutainer tubes fitted with handle and needles
8. Rack

Procedures

The test sera and the antigen will be left at a room temperature for half an hour every time before the test is proceeded.

9. 30 µl of each test serum will be taken and placed on a clean glass slide.
10. 30 micro liter of RBPT antigen will be added to the side of each test serum using

pipette.

11. Then the antigen and the test serum were mixed thoroughly by an applicator

12. The glass slide was shaken by hand for 4 minutes and

13. Finally the result of each test was read by looking the presence or absence of agglutination and the degree of agglutination was also appreciated in a very good light source.

Interpretation: After four minutes rocking (shaking) any visible agglutination was considered positive.

Annex 4: Complement Fixation Test procedure (CFT)

Procedure

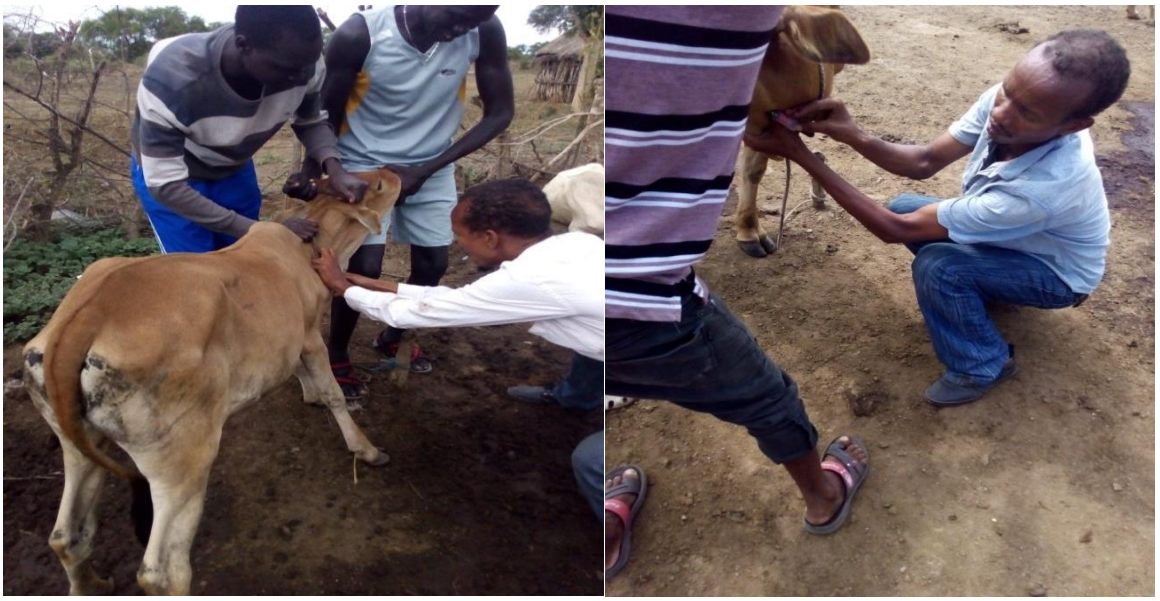
Test sera and appropriate working standards are diluted with an equal volume of veronal buffered saline in small tubes and incubated at 58°C for 50 minutes in order to inactivate the native complement. Using standard 96-well U-bottom microtitre plates, 25 µl volumes of diluted test serum are placed in the wells of the first and second rows, and 25 µl volumes of veronal buffered saline are added to all wells except those of the first row. Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the second row onwards continuing for at least four dilutions. Repeat steps ii and iii above for each serum to act as anti-complementary serum controls. Volumes (25 µl) of complement at 1.25 MHD, are added to each well and 25 µl of antigen, diluted to working strength, are added to all wells excluding those of the anti-complementary controls. These latter wells receive 25 µl of veronal buffered saline instead. Control wells containing: diluent only, negative serum + complement + diluent, antigen +complement + diluent, and complement + diluent, are set up to contain 75 µl total volume in each case. The plates are incubated at 37°C for 30 minutes with agitation at least for the initial 10minutes, or at 4°C for 14- 18 hours. Volumes (25 µl) of sensitized SRBC suspension are added to each well, and the plates are re-incubated at 37°C for 30 minutes with agitation at least for the first 10 minutes. The results are read after the plates have been left to stand at 4°C for up to 1 hour to allow cells to settle. For interpretation: Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 orat 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: Age determination in cattle based on teeth eruption

No	Teeth	Ages
1	I1 erupts	11/2-2 years
2	I2 erupts	2-2 1/2years
3	I3 erupts	3 years
4	C erupts	3 ½-4 years
5	All incisors are wear	5 years
6	I1 is level and the neck has emerged from the gum	6 years
7	I2 is level and the neck is visible	7 years
8	I3 is level the neck is visible	8 years
9	C is level and the neck is visible	9 years
10	The teeth that have not fallen out are reduced to small round pegs	15 years

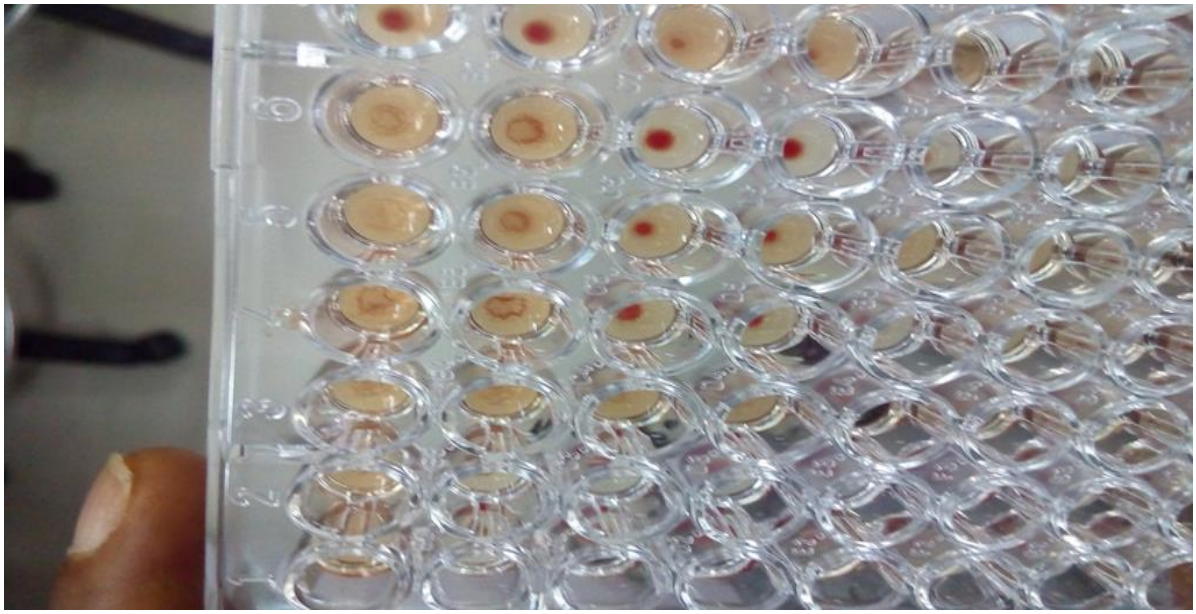
Source: Pace and Wakeman, 1983

Annex 6. Pictures showing different activities



Picture during blood collection

Picture during serum collection



Pictures during serological tests perfomed(RBPT and CFT)

Pictures of some herds