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COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

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SEROPREVALENCE, ASSOCIATED RISK FACTORS AND LIVESTOCK OWNER'S KNOWLEDGE, ATTITUDES AND PRACTICES TOWARD BOVINE BRUCELLOSIS IN SELECTED DISTRICTS OF ILUABABOR ZONE, SOUTH WEST OROMIA, ETHIOPIA

BY:

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Jimma University
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Seroprevalence, Associated Risk Factors and Livestock Owner's Knowledge, Attitudes and Practices toward Bovine Brucellosis in Selected Districts of IluAbabor Zone, South Weast Oromia Ethiopia

MSc Thesis

By:
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MSc. Thesis

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We, the undersigned, member of the Board of Examiners of the final open defense by **Mebratu Meles** have read and evaluated his/her thesis entitled **"Seroprevalence, Associated Risk Factors and Livestock Owner's Knowledge, Attitudes and Practices Toward Bovine Brucellosis In Selected Districts of Huababor Zone, South Weast Oromia, Ethiopia "**and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree Master of Science in **Veterinary Public Health**

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I declare that the thesis research works has not been done in study area else before or is not part of an on-going work.

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DEDICATION

I dedicate this research to God Almighty, my creator, for keeping me alive and healthy to see the completion of this phase in my life. Also, my dedication extended to my parents especially my father who was died and both of my mothers who were nurture all our family by sharing responsibility as husband and wife for parents. Special memory to my beloved Buse Bulti and my neonate child Biftu and my son Obsa and also I memories my brather Aschalew Meles because he encourage both my mother and all our family by his power and sharing responsibility.

STATEMENT OF AUTHOR

First, I declare that this thesis is my original work and that all sources of materials used for this thesis have been duly acknowledged. It has been submitted in partial fulfillment of the requirements for MSc. Degree at Jimma University, College of Agriculture and Veterinary Medicine deposited in the university library to be made available to borrowers under rules of the Library.

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BIOGRAPHICAL SKETCH

The author, Mebratu Meles Asfaw was born in Novenber 1974 E.C in Busano Kuni kebele Genji District, West Wollega Zone, Regional state of Oromia from his father Meles Asfaw, and his mother Werkenash Tegagne. He attended his elementary education from Grade 1 to 8 at Genji primary school 1982 to 1990 E.C, He had completed grade 9 to 12 Grades at Gimbi comperansive Secondary High school. Then, he joined Alage Agricultural Technical Vocational and Training College in 1998 E.C and graduated with Diploma in Animal health on June 28, 2000, E.C and then after graduation, he was employed by Genji district livestock and fishery development resource office as a rural animal health expert (Veterinary clinic) from August 2000 to 2008 E.C. He was joined again at Jimma University in 2009 and graduated with the Degree of Bachelor of Veterinary Science in June 2010. Then he continued his job as a rural animal health expert in August 2010 and appointed Veterinary clinic in 2011 as Genji District Livestock and fishery development resource office until he joined Jimma University, school of graduate studies for the degree master of science in Veterinary public health in October 2014 E.C.

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LIST OF ABREVATIONS

AOR	Adjusted Odds Ratio
CDC	Center for Disease Control
CSFPH	Center for Food Security & Public Health
CSA	Center for statical agency
C-ELISA	Competitive enzyme-linked immuosoret assay
CFT	Complemente Fixation Tests
CI	Confidance interval
COR	Crude Odds Raio
FAO	Food and Agriculture Organizaion
HRP	Horse reddish peroxide
IGM	Immunoglobulin type M
IHC	Immunohistochemistry
KAP	Knowledge, Attitude and Practice
KDa	Kilo Delta
LPs	Lipoplysaccharide
OD	Obtical Density
PBS	Tween (phosphate buffer solution)
PCR	Polymerase Chain Reaction
PH	Power Hydrogen
PI	Percentage Inhabition
RBPT	Rosebengal plate test
USD	Unite State Dollar
WHO	World Health Organization
WOAH	World Organization ForAnimal Health

ABSTRACT

Bovine brucellosis is a severe contagious disease that causes reproductive failure and has zoonotic potential with profound public health importance with widespread occurrence in developing countries like Ethiopia.The disease usually caused by *Brucella abortus*. Although much work has been done and reports are available in different parts of Ethiopia but there were limited information on the status of bovine brucellosis in the Ilu Ababor zone of the Mettu, Hurumu and Ale districts. The present study aimed to determined seroprevalence and the risk factors associated with bovine brucellosis seropositivity and appraise the livestock owners' knowledge, attitude, and practices related to brucellosis. A cross-sectional study was conducted from March 2023 to May 2024 on bovines under the traditional production system. Blood samples were collected from a total of 405 bovines to test the presence of Brucella antibody.Mulistage sampling was performed. The presence of antibody was tested using the C-ELISA. Risk factors for seropositivity were analyzed using multivariable logestic regressio n analysis. An overall 3.7% (95% CI: 2.3-6) and 11.6% (95%CI: 7.2-18.3) seroprevalence of brucellosis was recorded at animal and herd level respectively. The study identified introduction of new (OR=4.9, 95 % CI: 1.3-18.9), pregnant infect (OR=3.8, 95%CI: 1-14.5), abortion in herd (OR=4.6 95 % CI: 1.2-19.2). Repeat breeding (OR=5.4, 95 % CI; 1.4-21.4), large herd size (OR=4.4, 95% CI: 1.1-17.7) and herds kept with shoats were at higher risk (OR=5.3, CI; 95 % 1.6-17.7) of acquired Brucella infection. The results of this study demonstrated that bovine brucellosis is widely prevalent in the study areas**.** Most risk factors were management system related due to lack of enough knowledge about bovine brucellosis resulted to risky practices.Therefore, it is important to implement community education to increase public awareness about the transmission of brucellosis and further research should be conducted on brucellosis in the study area.

Keywords: *Brucellosis, cattle, C-ELISA, Ethiopia, seroprevalence*

1. INTRODUCTION

1.1. Background

Ethiopia has the largest livestock population in Africa according to the animal census, with an estimated 70 million cattle, 42.9 million sheep, 52.5 million goats, 8.1 million camels, and 57 million chickens (CSA, 2021). Livestock is a major source of animal protein, power for crop cultivation,means of transportation, export commodities, manure for farmland and household energy, security in times of crop failure, and means of wealth accumulation (Dessie, 2022) .

Despite the fact that Ethiopia has huge livestock resources, the production and productivity of the sectorre main low due to rampant infectious diseases, a traditional management system, a lack of infrastructure and unimproved genetic potential (Bifo *et al*., 2020). Among infectious diseases, brucellosis is a highly contagious, zoonotic, and economically important bacterial disease of animals worldwide (Bifo *et al*., 2020), which have propensity to adapt to new host and naturally transmitted to their primary hosts by contact (Khurana *et al*., 2021).

Among the 12 identified species ,*Brucella abortus* are the most important species exerting detrimental effects on both animal and human health (Asgari and Ahmadi, 2021). Brucellosis in bovines is predominantly caused by *B. abortus*, less frequently caused by *B.melitensis*, and occasionally caused by *B. suis* (Andrada *et al*., (2023). It is multiply and persist with in phagocytic cells of the host resulting in life time carriage of the organism (Adem and Duguma, 2020) .

Bovine brucellosis is the most important disease in many countries due to its high economic importance (Cárdenas *et al*., 2019a). Mainly affects sexually mature animals (Etefa *et al*., 2022). Characterized by premature abortions and breeding-related complications, such as repeat breeding, retained placenta, products of conception, metritis, stillbirths, weakness in offspring,reduced milk production in females, orchitis, and epididymitis in males (Shome *et al*., 2023). Hygromas, usually involving leg joints may be the only pathognomonic sign of the infection (Etefa *et al*., 2022) .

Bovine brucellosis is endemic in parts of Africa, Central and South America, Middle East and including Eshian in both humans and animals including in Ethiopia (Moriyón Uria *et al*., 2023). causing significant loss of productivity (Megersa *et al*. (2011a), as well as chronic and

febrile illness in humans (Etefa *et al*. (2022).Since the first report of livestock brucellosis in Ethiopia by Domenech, (1977), the disease has been noted as one of the important livestock diseases in the country (Etefa *et al*. (2022).

Management, animal movement, wide ranges of host, herd size and mixed farming of cows, buffaloes, sheep and goats has increased the risk factors for animal brucellosis where small ruminants act as primary hosts for *B. melitensis* and cattle as spillover host (Khurana *et al*., 2021). Bovine become infected with eating or drinking water, that is contaminated with the brucella bacteria , contact with uterine secretions or aborted fetuses; and through horizontal and vertical (Robi *et al*., 2024). *Brucella* reaches the placenta in females via hematogenous route and afterwards to the fetus. Abortion occurs due to the damage of placenta and due to stress induced hormonal changes (Khurana *et al*., 2021). Diseased animals shed the pathogen in uterine discharge, and milk (Abubakar *et al*., 2012) and these bacteria can spread with in the herd through ingestion of contaminated material (Arif and Thomson, 2023).

The possible risk factors for human brucellosis are feeding behavior, occupational exposure, contact with diseased animals secretion (Khurana *et al*., 2021). Approximately 50,000 human cases were annually reported around the globe (Afrin, 2021). Human brucellosis is popularly known as undulant fever,Crimean fever,Mediterranean fever, remitting fever, Maltese fever,goat fever, Gibraltar fever and bovine brucellosis is called as contagious abortion or Bang's disease (Afrin, 2021) .

Diagnosis of bovine brucellosis is based upon the isolation of *B. abortus* from abortion material, milk or necropsy material and serological responses to Brucella antigens can be demonstrated (Hariom and Dangi, 2021). The c ELISA was developed to overcome the cross reacts between vaccine strain S19 Gram negative and naturaly infected animals (Marageni *et al*., 2017). Treatment of brucellosis in domestic animals is unsuccessful (Manda, 2023) .Treatment of infected livestock is not attempted because potential problems related to maintaining infected animals in the face of ongoing eradication programs (Mitiku and Desa, 2020). Methods of prevention of bovine brucellosis mainly depend on health education to reduce occupational and food borne risks as well as elimination of the infection among animals through combination of vaccination of all breeding animals,elimination of infected animals or herds by segregation and slaughter (Mosiara, 2022).

Studies on the prevalence of bovine brucellosis have been carried out in many parts of Ethiopia by different researcher across various livestock production systems and breed. Ilu Aba Bora Zone is known for its high cattle production sector,but there is no information on the status of bovine brucellosis in selected districts of the Ilu Aba Bora zone of Oromia Regional State, Ethiopia, which gave impetus to the initiation of this study in current study area, Farmers's knowledge and awareness about brucellosis significantly reduces the sero-positivity of Brucella infection in animals (Banu *et al*., 2023). Thus,a cross-sectional study and competitive ELISA (cELISA) test were carried out to assess the status of seroprevalence of bovine brucellosis. Therefore, the objectives of this study were to estimate the seroprevalence of bovine brucellosis, identify potential risk factors that could precipitate the occurrence of bovine brucellosis, and to estimate the preciption of community.

1.2. Statement of the Problems

The Significance of Bovine brucellosis is known as an economically important zoonotic disease.Food safety is one of the principal pillars on which protection of human health resides; hence, laws, regulations, and veterinary policy measures alone did not bring the desired results. The whole community needs to be involved through health education in schools, in the workplace, and in the population at large (FAO) reported by (Okpala and Korzeniowska, 2023) If brucellosis-positive cattle enter the communal cattle population, there is a risk of spread between cattle. Community members are at risk of acquiring brucellosis through several high-risk practices, including the consumption of raw milk and the handling of cattle birth material reported by (Molnar and Godefroy, 2020) Developed countries have been able to control *Brucella* infection to a certain extent by implementing reliable diagnostic methods and usually culling animals that test positive for Brucella according to report of (Khurana *et al*., 2021). However, developing countries such as Ethiopia claim that the absence of compensation makes testing and killing unfeasible reported by (Rahman, 2015). Bovine brucellosis intracellular survival and adaptabile in the macrophages.Antibiotic treatment of brucellosis in domestic animals is unsuccessful. From research question, knowing releavance and prevalence of Bovine brucellosis in Ethiopia is a reference point to selected the present study title.Brucellosis is known to be an endemic and growing problem in domestic livestock herds in Ethiopia according to previous reports of (Tsegaye *et al*., 2016) (9.5%) in Arsi zone, and (Asmare *et al*., 2010) (13.7%) in Sidama zone, (26.1%) by (Megersa *et al*., 2011a) in

southern and eastern Ethiopia and (24.1%) by (Mekonnen *et al*., 2010) in western Tigray, **(**9**.**87%), by **(**Eticha *et al***.,** 2018**)** in Asella, (12**.**23%) by **(**Deresa *et al***.,** 2020**)** in Jimma zone Ethiopia.These report which analysis by a multivariable logestic regression model and associated risk factors at herd level.These work done by diifferrent diagnostic method including c-ELISA test.

C-ELISA is expected to reduce misclassification and increase the chance of detecting antibodies against brucellosis, to reducefalse positive and false negative in a given serum. Although much work has been done and reports are available in differrents parts of Ethiopia but there were no any previous information and published data on the status of bovine brucellosis in the Ilu Ababor zone of the selected districts (Mettu, Hurumu, and Ale districts) of the Oromia region of Ethiopia which gave impetus to the initiation of study in the current study area,In addition to seroprevalence finding this KAP study provides necessary information to address shortfalls in knowledge and practices within the study area to improve both animal and human health.Therefore, because of these, this study was conducted with the following objectives:.

1.3. Objectives

- To determine the seroprevalence of bovine brucellosis and its associated risk factors in cattle.
- To determine potential risk factors and their association with Brucella seropostivity.
- To assess the KAP of the community about bovine brucellosis transmission and effect on ecconomical and public health.

2. LITRATURE REVIEW

2.1. Etiology

Brucella *abortus* causes disease mainly in cattle reported by (Tulu, 2022). However,sheep, goats and other domestic animals can also be infected reported by (Edao, 2021). Gram negative, non-spore-forming and non-capsulated, partially acid-fast coccobacilli that lack capsules, They survive freezing and thawing but most disinfectants active kill Brucella. Pasturization effectively kills Brucella in milk.The bacterium is of 0.5-0.7μ in diameter and 0.6-1.5μ in length. Although Brucella species are described as non-motile, they carry all the genes except the chemotactic system necessary to assemble a functional flagellum reported by (Kiros *et al*., 2016) Brucellae come under α–2 subdivision of Proteobacteri reported by(Lopez-Goni and Moriyon, 2005). Within this phylum, there is the Alphaproteobacteria Class, encompassing bacteria with different shapes, lifestyles, metabolic capacities, and ecological variation (Suárez-Esquivel *et al*., 2020) .

2.2. Epidemiology

2.2.1. Geographical distribution

The disease is prevalent throughout the world except in Canada, Australia, Cyprus, Norway, Finland, the Netherlands, Denmark, Sweden, New Zealand and United Kingdom (Khurana *et al*., 2021).However,still common in Mediterranean ,Europe, Central and South America, Mexico, Africa, Near East countries, Central Asia, India and Italy are having significant prevalence of brucellosis (CDC, 2018) and (Zhang *et al.*, 2018), Bovine brucellosis was first reported in the African continent in Zimbabwe (1906) followed by Kenya (1914) and South Africa (1915) (Kiros *et al.*, 2016). However, the epidemiology of the disease in animals as well as humans is not well understood in sub Saharan countries of African continent (Samadi *et al*., 2024).

In Ethiopia, there is no documented information on how and when brucellosis was introduced and established. But the disease was reported for the first time in 1970s (Sohail *et al*., 2023). Since then,severals studies on the prevalence of brucellosis have been carried out in many parts of Ethiopia by different persons (Robi *et al*., 2023). In Ethiopia, seroprevalence of bovine brucellosis were reported in areas like Jimma Zone (3.1%), Central Oromia, (2.9%),

Arsi Zone (0.05%), Agro-Pastoral Areas of and Southern (3.5%) and Jijjiga (1.38%) East Wollega Zone (1.97 %), East Showa Zone (11.2%), Tigray Region,,Eastern Ethiopia & Guto Gida District (Jergefa *et al*., 2009), But in Ilu Ababor in selected districts no research done on bovine brucellosis and no this much information.

The extensive production system of the country is responsible for the mixing of different livestock speceis which facilitaties maintenances and transmission of brucellosis (Robi and Gelalcha, 2020). The evidences of brucella infection in Ethiopian cattle have been serologically evaluated in different parts of the country by differents authers (Table 1). There was high correlation for managemental factors like region, area, locality, size of the herd and knowledge regarding brucellosis and also factors related with animals like sex and age with seroprevalence of brucellosis (Awah-Ndukum *et al*., 2018). Several factors such as animal management, time difference, type and place of study may contribute for the high or less prevalence of the disease as presented (Table 1).

. ; , .

Table 1. Occurrence of Brucella infection in Ethiopia

Study	Number of	Bree	mgt	Prevalence	Type of Test	Reference
Borana	283	Local	Extensi	10.6	CFT	(Megersa et al., 2011a)
Centra	1136	Mixe	Intensiv	11	CFT	(Kebede et al., 2008)
North	384	Cross	Extensi	0.8	RBPT	(Pal et al., 2017)
West	1152	Local	Extensi	1	CFT	(Adugna et al.,
North	1968	Loca	Extensi	4.9	CFT	(Haileselassie et al.,
Assela	304	Mixe	Intensiv	14.1	RBPT	(Deselegn and Gangwar,
Ambo	169	Cross	Intensiv	0.2	RBPT	(Bashitu et al., 2015)
East	1106	Loca	Extensi	11.2	RBPT	(Dinka and Chala, 2009)
	423	Mixe	Extensi	4.3	CFT	(Robi and Gelalcha,
Debreze	300	Mixe	Extensi	2.0	CFT	(Alemu et al., 2014)
East	406	Mixe	Extensi	2.0	CFT	(Yohannes et al., 2012)
Sidama	370	Mixe	Extensi	0.1	CFT	(Asmare et al., 2010)

Mgts=management system

2.3. Host Range and Brucella Diversity

B.abortus is the principal Brucella organism that infects cattle.However,*B.suis* and *B.melitensis* may also infect cattle when they share pasture or facilities with infected pigs, goats and sheep (CFSPH 2009) and (Mosimane, 2019) .*B.abortus* has been reported from Yak according toreport of (Sun *et al.*, 2020).The main causative agent for brucellosis in dogs is *B.canis*; however, may be caused by *B.abortus*, *B.suis* and *B.melitensis* (Woldemeskel, 2013) .*B.abortus* isolated from the uterine discharge of female dog and cat housed together in a cattle farm (Khurana *et al*., 2021) . *B. melitensis* is principally responsible for brucellosis in goats .Camels could also be infected by *B. abortus* and *B. melitensis* (Khurana *et al*., 2021). *B. melitensis* and *B. suis* can be transmitted through cow's milk resulting in human infection (Akbarmehr, 2011).The changing geographical distribution of brucellosis a re-emerging zoonoses caused huge economic loss worldwide (Khurana *et a*l., 2021) .

2.4. Risk Factors of Bovine brucellosis

The epidemiology of the disease is influenced by several factors (Tulu *et al.,* 2020). Factors related to the host, the agent,the environment, and management practices determine the extent of exposure ,spread,and maintainance of brucellosis in a geographical area (Khurana *et al.*, 2021). Bovine brucellosis was transmitted from farm to farm through dogs responsible for carrying around aborted fetuses (Getahun *et al.*, 2022). Mixing herds at pasture and keeping the animals in shelters if in such areas parturition takes place,represent major factors for transmission (Addis and Desalegn, 2018). A significant association between brucella infection and risk markers such as abortion, retention of the placenta,repeat breeding,lack of clean water,insufficient manure removal and cleaning,poor management of aborted materials the introduction of new animals from herds that were unknown status,herds kepts in close confinement and mixed herds have reported by several reserchers (Tolosa, 2004).Animals breed with natural mating are reported to be more seropositive for brucella infection than animals bred with artificicial insemination (Addis and Desalegn, 2018, Deresa *et al.,* 2020) .

2.4.1. Host risk factors

Susceptibility of cattle to *B.abortus* infection is influenced by the age, sex and reproductive status of the individual animal. Infection occurs in cattle of all ages but persists most commonly in sexually mature animals (Sukumar *et al.,* 2012).Younger animals tend to be more resistant to bovine brucellosis infection and frequently clear infections ,although latent infections do occur and such animals may present a hazard when mature (Tolosa, 2004).This may be because sex hormones and erythritol, which stimulates the growth and multiplication of brucella organisms tend to increase in concentration with age and sexual maturity (Tilahun *et al*., 2022). Pregnant cattle are more susceptible to bovine brucellosis infection with the organism than sexually immature cattle of either sex.Susceptibility increases as stage of gestation increases (Hussen *et al*., 2020) .

2.4.2. Pathogen risk factor

B. abortus is a facultative intracellular organism capable of multiplication and survival within the host phagosome. Genome of *Brucella* is devoid of classical virulence genes which encode for plasmids, pili, exotoxins and capsules according to report of (Seleem *et al.*, 2008) These properties make lipopolysaccharide an important virulence factor for *Brucella* survival and replication in the host (Adem and Duguma, 2020) .

2.4.3. Farmers' Factors

Handling aborted material without appropriate personal protection and drinking raw milk are the major risk factors for transmission of brucellosis to human (Getahun *et al.*, 2023b) Awarenes creatation for the livestock owners on the effective implementation of sanitary and hygienic livestock management practice following abortion helps reduce spreading of bovine brucellosis the disease amongst animals as well as to the humans (Islam *et al.*, 2013b) .

2.4.4. Agro-ecological factors

Brucella abortus is sensitive to pasteurization, temperatures and its survival outside the hosts is largely dependent on environmental condition.The pathogen may survive in an aborted fetus in the shade for up to eight months,for two to three months in wet soil,one to two months in dry soil,three to four months in faeces, and eight months in liquid manure tanks (Mitiku and Desa, 2020).

2.4.5. Management risk factors

The spread of 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 (Mitiku and Desa, 2020) .

2.4.6. Occupational Risk Factors

Laboratory workers handling *Brucella* cultures are at high risk of acquiring brucellosis, abattoir workers, farmers and veterinarians are at high risk of acquiring the infection, feeding behavior (Dadar *et al*., 2023) .

2.5. Source of Infection and Mode of Transmission

2.5.1. In Human

Consumption of dairy products (especially raw meat,raw milk, soft cheese, butter and ice cream) is the most important source of infection (Radostits *et al.*, 2007a). Brucellosis, such as persistence in tissues of the mononuclear phagocyte system, including spleen, liver, lymph nodes, and bone marrow.Brucellosis has been reported as a risk factor for spontaneous abortion in women (Khan *et al.*, 2001), Treatment of brucellosis requires a combination of at least two antibiotics (including a tetracycline and an aminoglycoside or rifampin) to prevent relapses or complications (Khan *et al.*, 2001).

2.5.2. In Animal

Bovine brucellosis can be transmitted via horizontal or vertical route (Khurana *et al*., 2021) . The risk associated with exposure of susceptible animals to bovine brucellosis the disease following parturition or abortion of infected cattle depends on three factors; the number of organisms excreted the survival of these organisms under the existing environmental condition and the probability of susceptible animals being exposed to enough organisms to establish infection. The aborted fetuses, placental membranes and uterine discharges act as main source of bovine brucellosis infection (Khurana *et al*., 2021).The animals contract the infection by ingestion of contaminated feed and water or by contacting aborted fetuses, fetal membranes and discharges from uterus (Figure 1). Infected bulls spread infection by natural service or artificial insemination (Acha and Szyfres, 2001) .

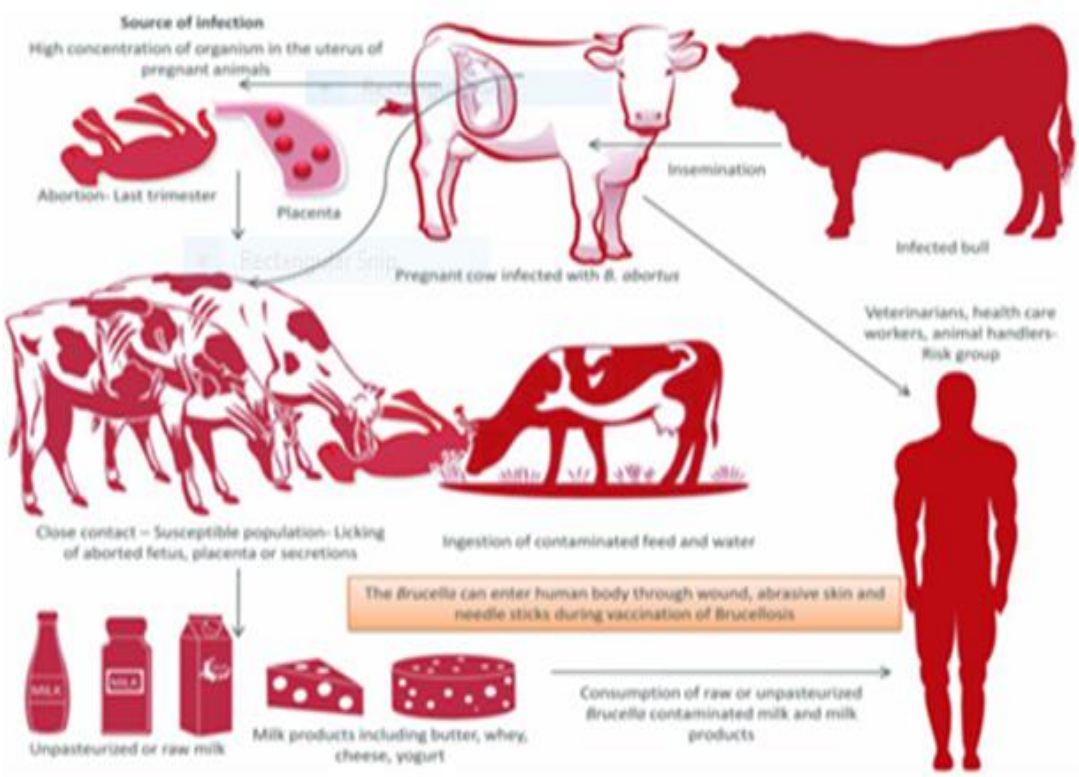

Figure 1: Transmission Bovine brucellosis from pregnant cows

Source;(Acha and Szyfres, 2001) .

2.6. Pathogenesis

Brucella infect the host and dissemination within the host (Christopher, 2010).The pathogen can survive in the macrophages and then multiply and control the fusion of phagosome–lysososme complex. *Brucella* could proliferate within the macrophages and escape from host defense mechanisms and can infect other host and also human host by contact with mucosa, puncture wounds such as needle sticks as well as ingestion. After that, the accumulated bacteria are circulated to other cells of host (Dadar *et al*., 2021). Virulence factor that activates the innate immune of in *Bovine abortus* system; are comprised of outer membrane proteins(Omps) (Im *et a*l., 2018). The bacteria are isolated commonly from genital organs and

associated lymph nodes in bulls. A large number of bacteria are excreted in semen during early acute phase, but the excretion decreases gradually later in chronic phase (Price, 1989).Brucella reaches the placenta in females via hematogenous route and afterwards to the fetus. The allantoic fluid factors in females stimulate the growth of Brucella, thereby making the uterus and reproductive tract of the pregnant female the site of the bacterial predilection. The elevated level of erythritol in the placenta and fetal fluid from fifth month of gestation is thought to be an important factor for abortion in animal. Erythrophagocytic trophoblasts localize in the placentome in vicinity of chorio-allantoic membrane resulting in rupture of cells and ulcer formation in the chorio-allantoic membrane. Abortion occurs due to the damage inflicated by the bacteria on the placenta and also due to stress induced hormonal changes (Kiros *et al*., 2016) .

2.7. Clinical Signs

2.7.1. Clinical signs in animals

The main manifestation in *B. abortus* infection being reproduction failure in the form of abortion and birth of weak offspring's which remain as carrier in herd. clinical .In highly susceptible non-vaccinated pregnant cattle, abortion after the 5^{th} month of pregnancy is a cardinal feature of the disease (Radostits *et al.,* 2007a). Calves could be infected at early stage (Khurana *et al*., 2021).The incubation period of bovine brucellosis could vary from weeks to months two together retention of fetal membranes, endometritis and reduction in milk production. Asymptomatic in young animals and non pregnant reported by (Kiros *et al*., 2016).The main clinical manifestations in males are severe epididymitis, orchitis and prostatitis (Meera, 2019) In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female (Khurana *et al*., 2021) . Abortion occurs after the 5thmonths of pregnancy; in bull, orchitis and epididmytis are cardinal signs. (CSFPH, 2008). Arthritis can develop after long-term infections. Even in the absence of abortion, there is heavy shedding of bovine brucellosis bacteria through the placenta, fetal fluids and vaginal exudates (Mitiku and Desa, 2020).

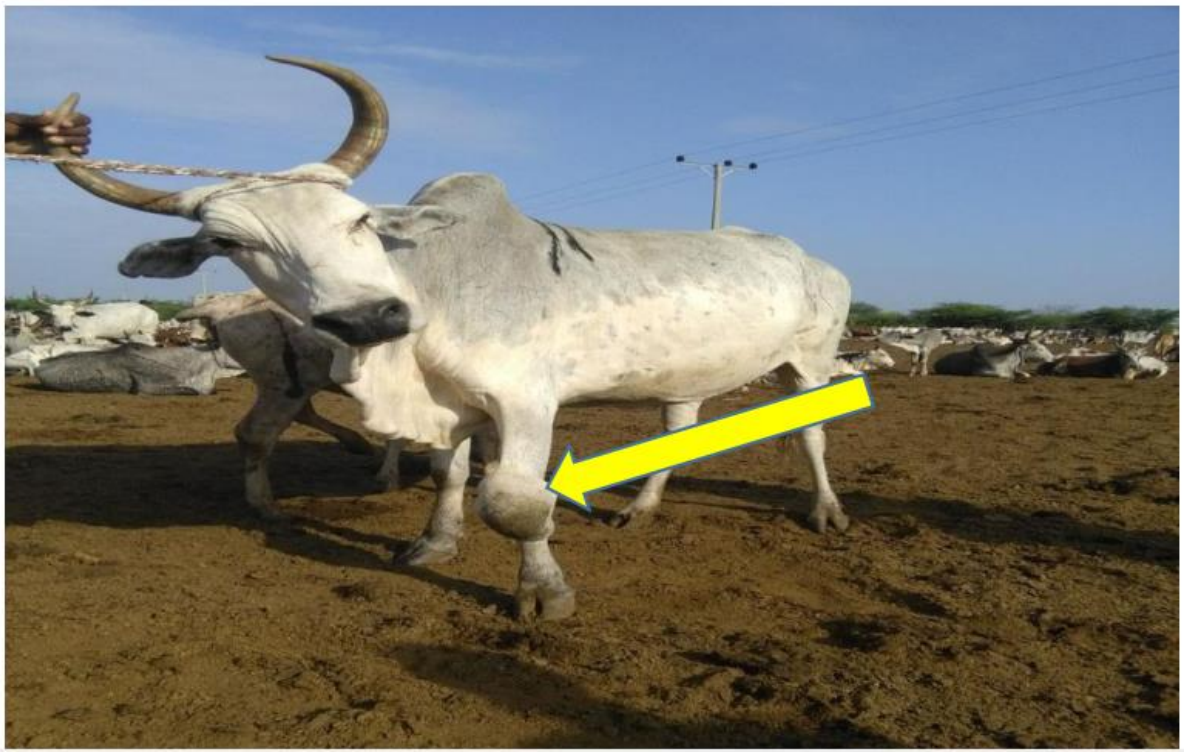

Figure 2: Characteristic hygromas in a cow

Source: (Wegi *et al*., 2021)

2.7.2. Clinical Signs in Humans

Range of non-specific clinical signs may be observed including with or without signs of localization, malaise, fatigue, sweats, anorexia, miscarriage headache, depression and abdominal or back pain (Lwoyero, 2009). The most common symptoms include undulant fever in which the temperature can vary from 37.8°C in the morning to 40°C in the afternoon; night sweets and weakness insomnia, anorexia, headache, constipation, sexual impotence, nervousness, encephalitis, arthritis, endocarditis, orchitis, and depression (Mitiku and Desa, 2020). The fever may mimic that of enteric fever and an undulant fever pattern is seen in chronic infections. Fever may be absent among patients with end-stage renal disease who acquire brucellosis (Sabra *et al*., 2021).Endocarditis is well documented including isolated case reports of Brucella infection of prosthetic valves, aortitis venous or arterial thrombosis, myocarditis and pericarditis (Willems *et al.*, 2022). Abortion is seen mostly in the first and second trimesters of pregnant women.Lack of appropriate therapy during the acute phases

may result in localization in various tissues and organs and lead to very hard to trea (Bosilkovski *et al*., 2020) .

2.8. Diagnosis

The isolation and identification for prevention, control of brucellosis, epidemiology and to monitor the progress of vaccination programs in cattle world health organization recommende d. Diagnosis (Kiros *et al*., 2016).The *Brucella* is frequently isolated from milk, supra-mammary lymph and iliac lymph nodes, spleen and uterus. However, bones, joints, brain and eyes might also become infected (Acha and Szyfres, 2001).

Specimen of fetal stomach, lung, liver, placenta, cotyledon and vaginal discharges are stained with Gram stain and modified Ziehl Neelsen stains. Blood or bone marrow samples can be taken cultured in 5-10% blood agar is used. To check up bacterial and fungal contamination; *Brucella* selective media are often used. On primary isolation it usually requires the addition of 5 -10% carbon dioxide and takes 3-5 days' incubation at 37^{O}C for visible colonies to appear. *Brucella* appears as small red-colored, coccobacili in clumps (Mequanent, 2022).

Direct Diagnosis of Brucella infection can be confirmed by demonstration of the bacteria in smears. In MZNstained smears, the Brucella organism appears as red intracellular coccobacilli or rod shapes,whereas other bacteria stain blue (Miguel López *et al*., 2011). .All Brucella strains are relatively slow growing. Incubation usually continues for 72 hours, but a negative diagnosis can only be made after a week-long incubation. Farrell's medium and Brucella albimi medium are selective enriched media for the isolation of Brucella species (Weldegebriall, 2015) .

Polymerase chain reaction (PCR) which is used as rapid detention, confirmation and differemtiating bovine brucella spp. This could be used for epidemiological interpretations and analysis as well as for molecular characterization (Habtamu *et al.*, 2013). Blood samples of naturally infected cows were found negative against *B. abortus*; however, milk and lymph tissues were found positive. PCR is also proven useful in diagnosing relapsing brucellosis, assessing treatment efficacy (O'Leary *et al*., 2006) .

Bovine brucellosis diagnosis and surveillance almost entirely rely on serological test used to detect antibodies against brucella antigens including lipopolysaccharides (LPS) and give indirect evidence of brucella infection (Adone and Pasquali, 2013) .

RBPT and LFA can be performed at the point of sample collection (Lucero *et al.,* 2003).The RBPT is a very sensitive test used for screening serum samples is quick, in expensive, and easy to implement. But it does not distinguish between field and S19 vaccine strain reactions (Londhe *et al*., 2011).False-negative reactions are rare but due to excessive heating in storage or in transit. The RBPT has a sensitivity of 96.10% and specificity of 99.30% (Tulu, 2022) .

The (CFT) is the most generally used test for serological confirmation and is recommended by WOAH (Alamian *et al.,* 2023). The CFT is based on the detection of particular antibodies of type IgM and IgG1 that fix complement (Nielsen *et al*., 2006). Such positive samples will be missed if they are only screened at a single dilution (Corbel, 2006).. The diagnosis of brucellosis in cattle may be adversely affected by the presence of cross-reactions that give false- positive serological test results due to S19 vaccine or other Gram-negative bacteria that share similar epitopes, such as *B. abortus* O-chain polysaccharides.Thus,Yersinia enterocolitic a 0:9, Escherichia coli O157:H7 (Adone and Pasquali, 2013).The ELISA can be used to detect brucella antibodies in milk or serum.The specificity and the sensitivity of the assay largely depend on the antigen, conjugate, substrate and the cut-of point used to establish infection (Al-Marzooqi *et al.,* 2022).

The i-ELISA has been used for smooth lipopolysaccharide and it is sensitive and specific for B. *abortus* or B. *melitensis*, but it is not capable of differentiating antibodies induced by the vaccine strains S19 or Rev1 (Lim *et al*., 2012) The sensitivity of the i-ELISA varies from 96% to 100% and its specificity from 93.8% to 100% (Padilla Poester *et al.,* 2010). Apparent diagnosis of Brucella infection or to screen a herd. Indirect and competitive enzyme-linked immunosorbent assays (WOAH, and CSFPH, 2009).

The c.ELISA, has is capable of reducing residual antibody formed in reaction to vaccination with S19. C-ELISA is a rapid assay, it is faster than CFT, it can be automated and therefore the results can be measured objectively .C-ELISA is its suitability for use on poor quality

affected by hemolysis (Seleem *et al*., 2008) higher affinity for the antigen than the vaccine or cross reacting antibody,but with lower affinity than the antibody arising from theinfection (Muñoz *et al.*, 2005).

The specificity of c-ELISA is very high and it is capable of identifying all antibody isotopes (Nielsen *et al*., 2006). Has high diagnostic specificity (100%) and a sensitivity of 98.8%, and it was observed to be the most specific test .The high specificity of c-ELISA is a result of its using particular monoclonal antibodies as a conjugate, which have the ability to connect with other non-specific antibodies and fix to certain specific epitopes on the smooth lipopolysaccharides antigen (Godfroid *et al.*, 2010) and (Di Febo *et a*l., 2012) .The only limitation of the c-ELISA is that it is cost to conduct than the screening tests (Díaz *et al.*, 2011).

2.9. Control and Prevention

Due to intracellular survival of Brucella and its adaptability in the macrophages, antibiotic treatment of brucellosis in domestic animals is unsuccessfull (Manda, 2023).Treatment of is infected livestock is not attempted because of the high treatment failure rate, cost and potential problems related to maintaining infected animals in the face of ongoing eradication (Mitiku and Desa, 2020). Humans are treated with antibiotics (doxycycline with rifamipicine). for brucellosis. However relapses are possible (Solís García del Pozo and Solera, 2012) However, rifampicin monotherapy is for treating brucellosis in pregnant women, and combined therapy of sulphamethoxazole and trimethoprim is recommended for children (Wareth *et al*., 2022).Investigation of the cause of abortion (Muma *et al*., 2007); training of livestock keepers Sammartino *et al*., (2005), test and slaughter of infected animals (Corbel, 2006). Vaccination is only practiced for diseases that have become endemic in a country (Aznar *et al*., 2017). Cattle vaccines contain either the smooth strain abortus S-19 or the rough strain RB-51(Olsen, 2013).Test and slaughter of positive animals if the herd is very low (Luelseged *et al*., 2018). Enriched biomedical research and commitment of government (Islam *et al*., 2013b). The prevention of human brucellosisis based on public health education to promoting public health and food safety (Corbel, 2006); collaboration between human and veterinary medicine

and Barrier precautions for hunters and professionals at risk (butchers, livestock owners, slaugheters,veterinarians) (Islam *et al*., 2013b).

2.10. Public Health and Economic Importance

Brucellosis a major public and animal health problem,where livestock are a major source of food and income (FAO,2003). Human brucellosis is primarily caused by B.*melitensis globally* . *B.abortus*, *B.suis* and *B.canis* also cause human brucellosis worldwide (Khurana *et al.*, 2021).Sheep, goats and their products are major sources of *B.melitensis* infection in human beings (Nagati and Hassan, 2016). It is mainly transmitted of *B.abortus* to human is as unpredictable as clinical manifestations (Kiros *et al*., 2016).Main source of transmission of *B.abortus* to human is through consumption of unpasteurized or raw milk or milk products including butter,whey, cheese, yogurt,ice-cream (Khurana *et al*., 2021).Pasteurization of milk before consumption is essential to prevent transmission of the pathogen to humans (Pal *et al.*, 2017). This poses apublic health threat to human unhygienic dairy farms where brucellosis is endemic (Khurana *et al.*, 2021).

Brucellosis in man is also considered as an occupational disease of dairy farmers, milking workers, dairy industry workers, slaughterhouse staff, butchers, hunters, shepherds, laboratory personnel and veterinarians (Mia *et al*., 2022). An estimate of overall economic loss caused by brucellosis, decrease in milk production, decrease in carcass weight, draught power, life expectancy, reproductive rates, market price, livestock population etc.) collected from published literature brucellosis caused a median loss of USD 3.4 billion to the livestock sector (Singh *et al*., 2015) extended calving interval in Sudan an average each sero-positive animal causes economic loss of USD 202 (Angara *et al*., 2016) .Increased awareness can reduce the level of Brucella sps.contamination in dairy products (Dadar *et al.*, 2021).Awareness creatation for community can reduce the human brucellosis.

3. MATERIAL AND METHODS

3.1. Study Area

This study was conducted in three districts (Hurumu, Metu, and Ale) of the Ilu Ababor Zone of Oromia Regional State, Ethiopia between the period of March 2023 and May 2024. Ilubabor Zone is located in the south-western part of the Oromia regional state, Ethiopia and 600 km away from Addis Ababa consists of 286 kebele and 14 districts and has one town administration, which has 23 kebele The zone is surrounded by Gambela Region in the west, in the east and southeast by SNNPS, in the north and west by Wollaga, and in the north-east by Buno Bedele. The Ilu babor zone comprises mostly gently undulating terrain with elevations ranging from 1,500m to 2,580 m, with latitudes of 7^0 40' 0'' N to 8^0 30 '0" and longitudes of 35^0 10'0 E to 35^0 50'0''E (Figure 3). The average temperature ranges between 16°C and 37°C, and the average rainfall is between 1,500 and 2200mm with most rainfall occurring from May to September (Singh, 2022). The prevailing wind direction is from the south-west during both the dry and rainy seasons (May to October) (Singh, 2022).

Out of them, this study was performed at three districts, namely Mettu, Hurumu, and Ale districts (Figure 3), which were predetermined by the Ilu Ababore zone livestock resource development office and Bedelle Regional Veterinary Laboratory Center management, depending on the monthly reports made by the respective veterinary clinics. Mettu district's located in the Illubabor Zone of the Oromia along the Sor River, has Area of the district: 68723 km^2. The boundaries are east Hurumu, south-west Bure, south-east Alle, south Bacho, north-west Darimu, and north Bilo Nopha district (Mettu District Livestock Resource and Development Office, 2021), (Hurumu District Livestock Resource and Development Office, 2023). Ale district is bordered on the south by the Southern Nations, Nationalities and Peoples Region, on the west by Nono, on the northwest by Bure, and on the northeast by Mettu. Gore Town is the Town of the Ale districts Ale District Livestock Resource and Agricultural Development Offce, 2023). Geographical description of districts present in (Table 2).

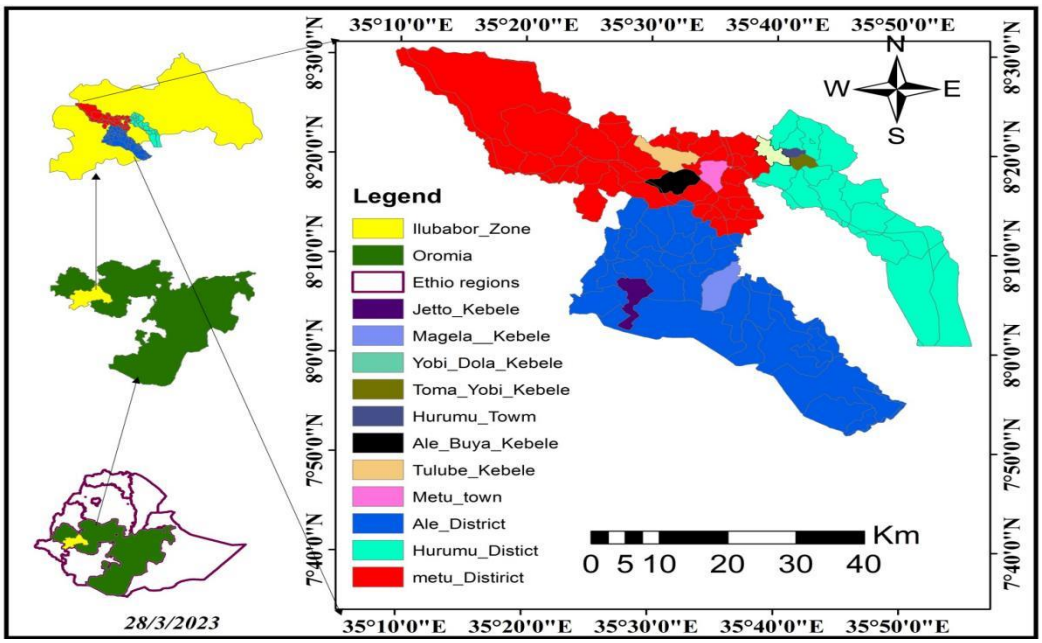

Figure 3: Map of study area

Table 2. Geographical charactestics information of study area districts

Geographical charactestics	Mettu	Hurumu	Ale
Latitude	8 ^{0}23’5’’N	8° 20’59’’N	8^{0}05’55-8^{0} 09’20’’N
Longitude	35 ^{0} 20 ‘31’’E	35° 39’10 " -35 ^{0}44’47’’E	35^{0}28’09-35^{0}3414’’E
Agro-ecology	83%wenadega, 17% kola,	86%wenadega, 9% kola, and 5% dega	only wenadage
Average rain fall	1204mm-1235mm	1500-2100mm	1900-2200mm
Altitude	1640-2027m	1693-1812m	1778-1992m
Average T^{o} ranges	24°C and 37°C,	16°C and 24°C	16°C and 24°C
Total number of householder	21807	9077	13962
Tota cattle population	239716	55300	91184
Number of kebele	31	14	24

Source; Data from Gps and districts

3.2. Study Design and Period

A cross-sectional study design was implemented to assess the sero-prevalence, associated risk factors, knowledge, attitudes and practices of owners toward bovine brucellosis in the study area.The study was carried out between the period of March 2023 and May 2024 .The Ilu Ababor zone of the three districts was selected purposefully, and a sample random sampling

strategy was employed for the sampling of the study villages, households (herds), and individual cattle.

3.3. Study Population

The target populations were cattle of different categories of breed, age, sex and parity kept under an extensive and sem-intensive management system in the study districts. As there is no history of vaccination against brucellosis in Ethiopia, all cattle older than six months were included in the study as the risk of the disease is not frequent in cattle younger than six months due to maternal antibodies in the sampling frame. The cattle under study were categorized into two age groups: young (6–24 months) and adults (>24 months), depending on their dentition, as categorized by (Parish and Karisch, 2013). Herd size was categorized into small (5–15), medium (16–30), and large (above 30 bovine) based on (Chand and Chhabra, 2013) Households that allowed blood sample collection from their cattle were used for the analysis of risk factors and knowledge, attitudes, and practices toward bovine brucellosis.

3.4. Sampling Methods and Sample Size

Due to the lack of previous study on brucellosis in cattle in the study areas, we used the formula provided by (Thrusfield, 2007) to determine the required sample size for this study. A desired absolute precision of 5% and a 95% confdence interval of the estimated prevalence of 50% were used in the calculation, resulting in a requirement of 384 cattle.However,a total of 405 blood samples collected from (Mettu,Hurumu and Ale distirct) of 137,148, and 120 respectivlly were sampled to increase the precision of the result. Hence, we oversampled by 5.5% of the computed number required based on (Naing *et al*., 2006).In this calculation, the versampled sample size by 5.5% was which are about 21 samples. Therefore, a total of 405 were sampled in the study.

$$n = (Z)^2 * \frac{Pexp\ (1 - Pexp)}{d^2} = (1.96)^2 * \frac{0.5(1-0.5)}{(0.05)^2} = 384$$

Where, n =sample size, z = statistic for a level of confidence at 95 % (1.96)

Pexp=0.5

We employed a multistage sampling procedure, with random selection of sampling units at each stage .The study zones and districts were purposefully selected because they had a large cattle population (unpublished data). kebeles, villages, and herds were selected using random sampling techniques. Specifcally, Ale Buya,Tullube,Mettu Town,Yoobi Doola,Tooma Yoobi Hurumu Town, Magella, Jeeto and Goree Town were chosen by lottery method from a total of 69 kebele in three districts From these districts, 9 kebeles were selected using a simple sampling technique. Based on the number of villages in the kebeles, villages were then randomly selected using a proportional simple random selection method. Finally, a simple random sampling method was used to select 129 herds based on the number of herds present in each village. For the sampling of individual cattle within each herd, a simple random method was used, with the number of animals sampled from each herd varying according to the number of cattle present.

3.5. Blood Sample Collection

For the evaluation of the prevalence of bovine brucellosis in the study areas blood samples were collected from cattle of which manage under (extensive 320 and 85 sem-itensive) management systems. 8–10 ml of blood samples were collected aseptically from the jagular vein of animals selected for serological examination by using plain vactunair tubes as recommended by WOAH (Afonso *et al.*, 2012). During the sampling, animals were restrained, and the area of the blood sample taken was first disinfected by using 70% alcohol before puncturing.The identification number of each of the animals was labeled on the corresponding vacutainer tube. The collected blood samples were kept overnight to allow clotting in a slant position at room temperature and then the sera were carefully decanted into 1.8 ml labeled cryovials without mixing with the clotted blood according to WOAH recomme nded (Afonso *et al*., 2012). Stored at veterinary clinics in the respective study districts.The harvested sera were then transported to the Bedelle Regional Veterinary Laboratory by using an icebox and stored at -20 °c until further processing was held.

3.6. Serological Test

The harvested sera samples were tested using a competitive enzyme-linked immunosorbent assay (C-ELISA) as indicated by the manufacturer. Initially, the washing solution was reconstituted as directed by the manufacturer or (WOAH) reported by (Perrett *et al*., 2010).

As there has never been history of vaccination in Ethiopia, seropositivity in all cases is due to natural infection PBS-Tween Soltion 20% Concentrate 1/20 was diluted in distilled water. Then, 500ml were prepared by adding 25 ml of PBS-Tween Solution to 475 ml of distilled water and mixing thoroughly.Test serums were added per well of the microtiter plate (wells) in addition to different solutions.4 ml of positive and negative control serum was added to the selected well coated with Brucella antigen.The plate was thoroughly shacked, sealed, and incubated at 37°C for one hour, then rinsed three times with PBS-Tween (phosphate buffer solution) buffer. 10 microliters of horse reddish peroxidase (HRP) conjugate were added to each well and incubated at 37 °C for one hour.

The plate was again rinsed three times, and a 10 microliter substrate solution (tetramethylbenzindine) was added to all wells.The plate was incubated for 10 minutes at 25 °C. Finally, the reaction was stopped by adding 50 microliters of stop solution to each well and mixing thoroughly. The obtical density (OD) of the control and test sample wells was measured at 450nm using a microplate photometer (Thermo Electron, Finland). The OD was measured in 15 minutes after adding the stop solution to prevent fluctuation in the OD values. All results were calculated in terms of pp (percentage positive) and interpreted accordingly. The sera sample showing a percentage positive value less than 30 was considered negative, and those showing a pp value equal to or greater than 30 were known as positive. All the samples were run in duplicate. In the present study. When the 405 serum harvested were tested according to the above procedure 15 (3.7%) were positive. Appendix. 4).

3.7. Statistical Data Analysis

All raw data were collected, the data were coded and entered into Microsoft excel spread sheet version 2010.Then the data were checked for any kind of error and correction proceded.Statistical package for social sciences (SPSS) version 26 (SPSSInc, IBM, Chicago, Illinois, USA) was used for statistical analysis of the data. Descriptive statistics like frequency and proportion were employed for the description of the prevalence of the disease and analysis of demographic characteristics of respondents involved in the study.A herd defined as the total number of cattle belonging to the same household and its may be included household one around family that their cattle grazing at one area and even the owners' diffirrent and their cattle grazed at only one site was considered seropositive if it included at

least one seropositive animal. Individual animal and herd level seroprevalence were calculated by dividing the number of positive test result by number of animals and herds sampled respectively. Odds ratio (OR) was used to measure the degree of association between risk factors. P-value less than 0.05 were considered statistically significant in all analysis.

Univariable analysis using chi square test was used for the analysis of the association between seropositivity and risk factors associated with the disease.In addition to the univariable analysis, a multivariable logestic regression model was used to analyze risk factors of the disease that was found statistically significant when using univariable analysis and the results were reported by odds ratio using 95 % confidence interval to assessed the strength of the association.Multivariable logestic regression model selection was based on p-value $\leq$0.25 Bashitu *et al*., (2015) and backward elimination procedure. The statistical significance level was set at 95% confidence level and 5% level of precision So that p-value $\leq$0.05 was considered significant. The model validity and prevdictive ability were assessed using the Hosmer-Lemeshow test. Knowledge, attitudes and practices of this study participant were analysed using descriptive statistics and frequence of presentage showing their proportion were used.

3.8. Survey for Risk Factors Evaluation

For investigation of the prevalence of bovine brucellosis and its determinant factors, general identification such as: specific location of the animals (districts and villages); breed (local and cross), age of animals (young and adults); herd size (<15,15–30,>30); parity (monoparous and multiparous); reproductive category (bull, heifer cow); management system (semi-intensive and extensive); Introducing new animals into a herd (yes and no), types of service for breeding (AI and natural bull), status of pregnancy (pregnant or not pregnant), history of abortion (present or not present), history of retained fetal placenta (present or not present), and history of repeat breeding (present or not present) of the selected animals were documented. For this study, 14 pre-test semi-structural questionnaire surveys were answered by all eligible households whose animals were included in the sampling unit, irrespective of gender and educational status, to investigate risk factors for the occurrence of bovine brucellosis in the study areas (Appendices 1).

3.9. Questionnaire Survey for Assessment of KAP

In the history of Ethiopian civilization, agricultural development has undergone a series of evolutionary changes in crop and livestock production (Babikir *et al.*, 2015) This practice creates a good opportunity for rural communities to improve their lives but might also pose a threat to animal and public health if zoonotic pathogens like Brucella species are present. Improvement of knowledge,attitudes and practice (KAP) among mixed crop-livestock farmers could have an important impact on the reduction of many zoonotic infections, including bovine brucellosis (Olaogun *et al*., 2023). Therefore for this study, 25 a standard structured, pre-tested questionnaires were presented to respondents to obtain general information on the potential risk factors associated with the occurrence of bovine brucellosis in the study area. First, the questionnaire survey was prepared in English and translated into the local language (Afan Oromo) for the assessment of both KAP and risk factors. From one district, three kebele were selected by simple random sampling, and from three districts, nine kebele were selected.The objective of the survey was explained to them before the start of the interview. For this, verbal consent was obtained from respondents, from which their animals were tested for brucellosis.

Demographic features like gender (male and female), age, level of education (illiterate, basic education, primary school, secondary level, diploma, degree, and above), place of residence (village and district), marital status (married, unmarried, divorced, and widowed), previous knowledge of the disease (yes, no), in general perception, and their daily activities toward bovine brucellosis were recorded. Husbandry questions (species kept, number of livestock, history of abortions and stillbirth, contacts between livestock, the source of their cattle, awareness of the respondents on the risk of transmission of brucellosis from cattle to cattle and cattle to humans, management practices, aborted material disposal practices, handling of retained placenta and household consumption habits of raw milk and raw meat, knowledge about the disease, how to assess their cattle during delivery. The questionnaire was administered to those selected individuals and the required informations were collected. Then the knowledge, attitudes, and practices of the livestock owners' toward the disease were evaluated to assess their adherence to control measures. (Appendices 2 and 3). The total

sample size of the households was determined by using the formula recommended by (Arsham, 2007) for the KAP analysis.

That is, $N = \frac{0.25}{(SE)^2}$

Where N = required sample size and

SE = standard error

Depending on this formula, the total number of respondents who participated in the study was calculated by assuming the standard error of 22.5%, just to increase the number of participants, the precision level at 5%, and the con**fi**dence interval of 95%; then, 129 participants were selected from all the nine villages ofthe study district for KAP interview

3.10. Limitation of the Study

Blood samples examined in this study did not utilize screening test like Rose Bengal Plate test due to inaccessibility of the kit to the required amount so that C-ELISA was used for all of the samples.

3.11. Inclussion and Exlusssion Crateria

All Above six month age and only health bovine were included .Bovine under six month exluded.Regarding protocol we used negative and positive control chemical to prevent false positive and false negative.

3.12. Ethical Clearance

The study was approved by the research ethical committee and and the Letter of clearance of the protocol for field studies and collection of animal materials was approved and taken by the animal research ethical review committee of the Jimma University College of Veternary Medicine and Agiculture (CVMA) with certificate reference number A 17/May/2024 . Bedele Regional Veterinary Laboratory Office supports me for sample collection and processing. For the questionnaire survey, pastoralists were informed of the aim of the study and their verbal consent was sought before the commencement of data collection (Appendix 1-3).

4. RESULTS

4.1. Over all seroprevalence of bovine brucellosis

The overall sero-prevalence of bovine brucellosis in the present study areas was found to be 3.7% (CI: 2.3–6) and 11.6% (CI: 7.2–18.3) at animal and herd levels, respectively, from which the highest prevalence was detected in Hurumu district with a proportion of 5.4%, the medium sero-prevalence was detected in Mettu District at 3.6%, and the sero lowest prevalenc e was detected in Ale District at 1.66%. (Table: 3). A statistically significant difference ($P < 0.05$) was found between study areas at both the animal and herd levels (Table 3).

Table 3 .Results of C-ELISA across study districts and village

Study area Districts	Towns and villages	Animals level seroprevalence				Herd level serprevalence			
Mettu		Total test	Positive (%)	P.Value	CI:95%	Total test	Positive(%)	P.Value	CI:95 %
	Ale Buya	52	4(7.6)	0.3	3-18.2	15	4(26.6)	0.2	0.5-52
	Tulube	44	1(2.3)	0.9	0.4-12	15	1(6.6)	0.8	1.7-24.6
	Mettu Town	41	0	0	0	13	0	0	0
Over all result		137	5(3.6)	0.34	0.5-11.7	43	5(11.6)	0.13	5.1-24.5
Hurumu	Yobi Dola	54	3(5.5)	0.43	2-15.1	15	3(20)	0.3	0.3-38
	Toma Yobi	54	3(5.5)	0.43	2-15.1	15	3(20)	0.3	0.3-38
	Hurumu Town	40	2(5)	0.52	1.4-16.5	13	2(15.4)	0.47	0.3-32
Over all result		148	8((5.4)	0.13	0.7-16.2	43	8(18.6)	0.04	9.7-32.6
Ale	Magela	40	2(5)	.0.52	1.4-16.5	15	2(13.3)	0.55	2-26
	Jetoo	40	0	0	0	15	0	0	0
	Goree Town	40	0	0	0	13	0	0	0
Over all result		120	2(1,66)	0.3	0.5-6	43	2(4.6)	0.2	1.3-15.5
Over all total		405	15(3.7)	0.3	2.3-6	129	15(11.6)	0.013	7.2-18.3

4.2. Animal-Level Risk Factors of Brucellosis

In present study, the results indicated a insignifcant association between age,Sex,breed groups, management system, types of service and the prevalence of brucellosis ($P > 0.05$). Animals which showed seropositive had a older, age, local, breed, cattle under extensive management, bovine which use natural bull types of Service for breeding and most female animals were showed *Brucella* seropositivity and no any of the infected from those use AI, cross breed and under semi-intensive management. The study found a signifcantassociation ($P< 0.05$). Multivariable logistic regression analysis between introducing new animals into herds ,herd size,a history of abortion, multiparity animals, status of pregnancy, in retained placenta,history of repeat breeding, and seroprevalence of brucellosis.The study found a history of abortion in cattle. With an OR of 4.9 when compere with animals which not have history of abortion.The study found a in multiparity animals with OR 5.8 than monoparity. Pregnancy between brucellosis seropositivity with OR 6.8 than animals which no have status of pregnancy The study also found with OR 7.5 than animals which no have history of retained placenta.The study found between brucellosis seropositivity and in repeat breeding with OR 4.9 than animals which no have history of repeat breeding. All these showed that a significant association ($P<0.025$) and ($p< 0.05$) were univariable and multivariable logistic regression analysis respectively. However, breed, age, sex, types of service,management system and location (urban and rural) not showed significantly associated at univariable analysis ($P > 0.05$) with the seroprevalence of brucellosis (Table 4).

Table 4. Animal-level risk factors of bovine brucellosis

Variables	Category	Total animals		Univariable			Multivariables		
		Test	Positive (%)	P-value	OR	CI ;(95%)	P_value	OR	CI;(95%)
location	Urban	120	2(1.7)						
	Rural	385	13(3.4)						
Age	Young	104	0						
	Adult	301	15(5)	0.9					
Sex	Male	79	2(2.5)						
	Femals	326	13(4)	0.54	1.6	(0.4-7.0)			
Breed	Cross	32	0						
	Local	373	15(4)	0.9					
Pregnant	Not present	192	5(2.6)						
	Present	52	8(15.3)	0.001	6.8	(2.2-21.7)	0.049	3.8	(1.1-14.5)
Abortion	Not present	193	6(3)						
	Present	51	7(13.7)	0.00	4.9	(1.6-15.4)	0.023	4.8	(1.3-18.5)
Parity	Monoparity	150	3(2)						
	multiparous	94	10(10.6)	0.00	5.8	(1.6-21.7)	0.024	5.6	(1.3-25)
HRP	Not present	182	4(2.2)						
	Present	62	9(14.5)	0.001	7.5	2.3-25.5	0.031	4.3	(1.2-16.3)
HRB	Not present	179	5(2.8)						
	Present	65	8(12)	0.007	4.9	(1.5-15.5)	0.004	6.9	(1.8-26)
Types service	AI	41	0						
	Natural or bull	203	13(6.4)	0.9					
Introduction	Born	328	6(1.8)						
	Bought	77	9(11.7)	0.00	7.0	2.5-20.6	0.001	6.5	2.1-19.0
Herd size	Small	156	3(1.9)						
	Medium	168	5(3)	0.55	1.5	0.4-6.0			
	Large	81	7(8.6)	0.02	4.8	1.3-19.0	0.045	5.4	1.1-27.7
Mgs	Semi-intensive	85	0						
	Extensive	320	15(4.7)	0.9					

HA=History of abortion, HRP=History of Retained placenta, HRB=History of Repeat breeding, HPS= History of Pregnancy status, OR: Odds Ratio; CI: Confdence Interval

4.3. Herd-Level Risk Factors of Bovine Brucellosis

Herd size, introducing new animals, abortion in herd, and Species composition with in herd and Contact between other herd were found to have a signifcant effect (P <0.05) on the herd-level seroprevalence of cattle brucellosis in both univariable and multivariable analyses. However, the analysis showed that there was no signifcant association (P > 0.05) between the herd-level seroprevalence of brucellosis and factors such as breed, and management system, as shown in (Table 5).

Table 5. Analysis of potential risk factors for brucellosis at the herd level using univariate and multivariate methods

Variables	Category	Total herd tested	Totalherd positive (%	Univariable		Multivariable	
				P value	Crude OR (CI 95%)	*P* value	Adjusted OR (CI 95%)
Herd size	small	55	3(5.4)				
	medium	49	5(10.2)				
	large	25	7(28.0)	0.01	6.7(1.6-	0.046	5.1(1.1-25.0)
Species	Only cattle	65	3(4.6)				
composition with	Mixed with	64	12(18.7)	0.02	4.7(1.3-	0.034	4.3(1.2-16.4)
Introducing new	no	92	6(6.5)				
animals	yes	37	9(24.3)	0.007	4.6(1.5-	0.015	5.3(1.4 -20.3)
Abortion in herd	No present	103	8		4.3(1.4-		
	present	26	7	0.0000	5.3(1.7-	0.006	5.7(1.7-19.0)

4.4. Potential Risk Factores and Their Association with Brucella Seropositivity

4.4.1. Animal-associated risk factors

Animal-associated (adult, most of them female, most of them multiparious cows, all infected local breeds, most of them cows which have history of (retained placenta, abortion and pregnant) were showed seropositivity to bovine brucellosis. Regarding the status of pregnancy and reproductive category, 8(3.3%) seropositive animals were pregnant and 2(2.5%) and 13 (5.33%) seropositive animals were bull and cows respectively. However, none of the young animals, cross breeds and heifers was found seropositive. On the other hand, status of

pregnancy, history of abortion and history of repeat breeding, history of retained placenta, parity were found statistically significant by multivariable logistic regression model (P-value <0.05 (Table 4).

4.4.2. Management related risk factors on seroprevalence of bovine brucellosis

Animal management-related risk factors include breeding practices, herd size and level of farmer awareness about the disease, husbandry practices, extensive production system, introduction of new animal into herd, contact with other herd, grazing by mixing with different species like (shoat), lack of isolation shed for sick animal, lack of pour water and animal which have large herd density showed seropositivity .

Univariable logestic regression analysis of management risk factors and seroprevalence indicated that all sero-positive animals were managed under extensive management system. In which 3 (2.3%) and 5(3.9%) and 7(5.4%) showed sero-positive animals were from small , medium and large herd sizes respectively. The feeding style of 11 (8.5%) of sero-positive animals were mixed with other herds. More than half which showed sero-positive animals 9 were from the market whereas 6 of them were from both market and own source.

Out of the total sero-positive animals, 8 were from the herds using both natural service for breeding and artificial insemination. 7 animals which showed seropositivity from herds which use natural bull for breeding system. Likewise, no any one infected from herd which use tap water from town. All 13 of bovine showed seropositivity which use management at rural area which use any available water and surface water because which more contaminated by any animal urine and feces. However, management (extensive and semi-intensive) system and source of water found not statistically significant by univariable logistic regression analysis (p>0.05). All of management related risk factors was found statistically significant by (univariable logistic regression analysis (P-value <0.025). Thus it needs to subjected to multivariable logistic regression analysis.(Table 6).When subjected to Multivariable logistic regression analysis showed statistically significant association.(P-value <0.05).

Table 6 .Management related risk factors by univariable and multivariable logistic regression analysis

Factors	Catagory	No of animals tested	C.ELISA	Univariable			Multivariable		
				P.value	OR	CI:95%	P.value	OR	CI:95%
Management system	Semi intensive	27							
	Extensive	102	15						
Herd size	small	55	3						
	medium	49	5						
	large	25	7	0.01	6.7	1.6-28.9	0.032	5.2	1.2-23.2
Contact withother herd	no	115	9						
	yes	14	6	0.001	8.8	2.5-31	0.035	4.4	1.1-17.5
Feeding style	separated	79	4						
	Mixed with other spp	50	11	0.007	5.3	1.6-17.7	0.034	4.3	1.2-16.4
Source of replacement stock	born	82	6	0.007	4.6	1.5-14.1	0.02	5.3	1.4-20.3
	bought	47	9						
Types of Service	Natural bull	107	8						
	AI	22	7	0.003	5.7	1.8-18	0.02	4.9	1.4-18.6
Source of water	Tap water	27	0						
	Surface water	102	15						

4.4.3. KAP Assesment

Knowledge of respondents on Bovine brucellosis

Out of 129 participants, 32 (24.8%) indicated they had heard of brucellosis previously.The main sources of information on brucellosis were veterinary services (90.6%; 29/32), community gatherings or talks (3.1%; 1/32), and radio or television (6.2%; 2/32). Regarding which animals could become infected with *Br.Abortus*, 71.8% (23/32) of them stated as cattle, 6.3% (2/32) sheep and goats and 21.8% (7/32) all animals. Knowledge about the transmission of brucellosis in cattle, 87.5% (28/32) of respondents know and said that transmited by pasture and water contamina**t** by abortion. Participants reported the following as signs of brucellosis in cattle**:** 62.5% (20/32) abortion and 15.6 %(5/32) weak calves,

whereas 21.9% (7/32) of respondents retained fetal placenta know. Only 96.8% (31/32) of respondents knew that humans could become infected with brucellosis. Among these respondents**,** drinking raw milk (12.5%; 4/32) and handling an abortion with bare hand (84. 4%; 27/32) were reported as possible means of zoonotic transmission 93 %(31/32) respondents responsed that Bovine brucellosis is preventable by 12.5 %(4/32) avoiding drinking of raw milk and 84.4 %(27/32) avoiding of bare hand handling of abortion (Table 7).

Table 7 . Knowledge of respondents' towards brucellosis prevention

Knowledge of brucellosis	catagory	Number of respondent	Percentage (%)
Raring of bovines	yes	129	100
		0	0
Do you know a disease called bovine brucellosis	yes	32	24.8
	no	97	75.2
From which sources of information you heard ?	Vet.service	29	90
	Radio and television	3	10
Which livestock species are affected by brucellosis ?	cattle	23	71.9
	All animals	7	21.9
Clinical signs of brucellosis in cattle	abortion	20	62.5
	retained fetal placenta	7	21.9
	Born weak calves,	5	15.6
Does it transmit from animal to animal?	yes	31	96
Mode of transmission b/n animal	Pasture and water contaminat by abortion.	28	87.5
	By contact during communal grazing	4	12.5
Does it transmit from animal to human?zooonotic	yes	31	96.8
Mode of transmission to human	Handling aborted material with bare hand	27	84.4
	drinking raw milk	4	12.5
Can preventable ?	yes	31	96.8
?mechanism of prevention	Avoiding of handling aborted material with bare hand	27	84.4
	Avoiding drinking raw milk	4	12.5

Attitudes to wards Bovine brucellosis

Respondents had reflected positive attitude towards some questions. (93%; 120/129) stated that they would like to receive more information on brucellosis. 50/129 from public health professional 70 /129 from veterinary.service**.**To ensure cattle health, 10% (13/129) of respondents stated that they sought veterinary advice 12.4% (16/129) bought from people they knew or trusted and 45.7% (59/129) relied on their own experience (Table 8).

Table 8 .Respondents' attitude (positive attitude) towards brucellosis prevention

Parameter of attitude questions	Catagory	Totalofrespondents	Totalofrespondents
Do you believe that family members working most with cows exposed to brucella infection are at high risk of infection?	Yes	32	24.8
	No	97	75.2
Would you like to receive more information on brucellosis?	Yes	120	93
	No	9	7
From which source do want the information	vet.service	70	54.3
	Public health profetional	50	39
To ensure cattle health**,** who would you sought to bought?	vet.service	13	10
	Own exprince	59	45.7
	Bought from people	16	12.4

Practice of respondents' towards brucellosis prevention

From the present findings, most (85.5%) of the respondents consumed raw milk.71 % of respondents handled aborted fetus and placental memberans with bare hand. only 14.7 % use glave and 13.9 % washing their hand after through (Table 9) .

Table 9 . Practice of respondents' towards brucellosis prevention

Practice of respondents	catagory	Totalrespondents	Percentage (%)
Do you drink raw milk and its product?	Yes	109	85.5
How do you handle abortedfetus and placental memberans?	Bare hands	92	71.3
	using gloves	19	14.7
	washing after throgh	18	14
What do you do if aborted fetus found?	Burial	23	17.8
	Through aways	106	82.2
What do you do if placental memberans found	Through away to deep	87	67.4
	give to dog	33	25.6
	Burial	9	7
Who personnel assissting your cattle during delivery?	Veterinarian	17	13.2
	Shepherds	15	11.6
	house hold members	97	75.2
Mixed differrent spp like shoat and bovine	Yes	92	71.3
	No	37	28.7
AI service for animals breeding	yes	33	25.6
	no	96	74.4

5. DISCUSSIONS

This study revealed that various factors at the individual animal level, such as age, sex, abortion, parity, history of repeate breeding, species composition, herd size, management practices, and introduction of new animals, can affect the prevalence of brucellosis in cattle. Moreover, the study found that herd size, introduction of new animals, species composition, repeate breeding in herd and, abortion in herd signifcantly influence the prevalence of Brucella infection at the herd level. The high prevalence of Brucella infection in cattle in the study areas causes severe economic losses due to reproductive disorders such as abortion, calving interval due to repeat breeding, treatment cost due to retained placenta and infertility, and also poses a signifcant risk to public health.

In the present study, the overall seroprevalence of Brucella antibodies at the animal level was found to be 3.7%. This aligns with previous reports 3.65% by (Waktole *et al.*, 2018) in Selected Dairy Farms in Bishoftu Town, Oromia, Ethiopia, 3.5% by (Megersa *et al*., 2011b) in Southern and Eastern Ethiopia. Similar findings were also reported from other African countries such as in Central African 3.3% by (Nakoune *et al.*, 2004) , (3.5%) by (Angara *et al*., 2004) in Kuku Dairy North Sudan and from cattle selected in different areas in Cameron 3.4% by (Awah-Ndukum *et a*l., 2018). However, the prevalence reported in this study is lower than studies conducted in the country, such as (11.0%) in (Wuchale-Jida district) by (Kebede *et al*., 2008) ,(10.6%) from Borana by (Edao *et al*., 2020) and 14.1%) from Assela by (Deselegn and Gangwar, 2011). However, higher seroprevalence have been observed in other countries by other authors including Uganda (46.8%) reported by (Kungu, 2011) and Togo (41%) by (Domingo, 2000). In the present study the higher seroprevalence was recorded in Hurumu district as individual and herd level.

The current study's seroprevalence results were higher than those reported (1.5%) by (Tesfaye *et al*., 2011) from Addis Ababa dairy farms and (2.6%) by (Tsegaye *et a*l., 2016) from Arsi zone. The variation in seroprevalence across different study areas and countries could be attributed factors, including environmental conditions, clinic and accessibility to veterinarian, management strategies,awareness of the community,sources of replacement animals, hygienic practices on the farms(Radostits *et al*., 2007b).

The herd-level seroprevalence in this study (11.6%) is similar to that reported by previous studies, such as (Robi and Gelalcha, 2020) (11.6% in the Jimma zone), (Etefa *et al*., 2022), (12.3% in Jimma zone), (Tsegaye *et al*., 2016) (9.5% in Aris zone), and (Asmare *et al*., 2010) (13.7% in Sidama zone). However, it is lower than the findings reported (24.1%) (Mekonnen *et al*., 2010) in western Tigray Ethiopia .Other studies have reported even higher herd-level seroprevalence, such as 62% in Zambia (Muma *et al*., 2007) 55.6% in Uganda (Wolff *et al*., 2017), Herd level seroprevalence of the present study was higher than the findings reported of 2.96% by (Tolosa, 2004) in Jimma zone and 4.9% by (Adugna *et al*., 2013) in western Ethiopia. The difference in results could be due to variations in the prevalence of the disease at the overall animal level and the herd size at the time of the study. Moreover, the study found that the size of the herd was a risk factor for *Brucella* seropositivity at the herd level, with larger herds having approximately five times higher odds (OR = 5.4) than smaller herds. This finding is consistent with report of (Megersa *et al*., 2011a) and (Tulu, 2022) who reported that large herds in southern and eastern Ethiopia were at higher risk of acquiring Brucella infection at the herd level respectively.

The variation in seroprevalence across different study areas and countries could be attributed to various factors, including environmental conditions, management strategies, farming practices, sources of replacement animals, farmer education levels and hygienic practices on the farms (Radostits *et al*., 2007b).

Based on serological evidence, this study provides epidemiological data on *Brucella* infection in cattle in Ilu Ababor zone Oromia regional state, Ethiopia. As the first study in the selected areas to provide information on the epidemiology of brucellosis, this research could inform the application of appropriate management techniques to control and prevent the disease in cattle.

In present study, 13 of bovine showed seropositivity which use management at rural area this may be due to most of cattle tested in the present study was from urban is which managed at semi-intensive this may propability to exposure low due to graize after dew drop and use tap water and rural animals use any available surface water.

According to the present study, all infected animals over six years old. This finding is in line with (Sagamiko *et al*., 2018) report higher in cattle aged 6-10 years old. Young animals could

be attributed to resistance of sexually immature cattle to infection accoding to report of (Robi *et al*., 2023). It is essentially a disease of sexually mature disease (Getahun *et al*., 2023a) .

Females which showed bovine brucellosis seropositivity (AOR=1.6) times more likely showed seropositivity than males. This observation was in agreement with the work of (Yuguda *et al*., 2019) who reported only female reactors Sex has been one of the risk factors affecting susceptibility of cattle to *B.bortus* infection very high in females due to hormones which promote bacterial growth and multiplication present in reproductive tract females than in males (Radostits *et al*., 2007a).

All of the seropositive cattle in the current investigation were native breeds. In line with (Robi and Gelalcha, 2020) which reported (OR=4.5) more likely to be acquired Brucella infection than cross breed In contrast to the present study (Deka *et al*., 2018) and (Tulu, 2022) reported a higher incidence bovine brucellosis in exotic and cross-bred animals than in native cattle. In the present study the variation may be due to variation in the breeds of animals sampled, management practice and herd size.

Pregnant cows in present study has 3.8 times more likely to be seropositive than nonpregnant cows. This finding is in agreement with the report of 4.3 (Haileselassie *et al*., 2011), Etefa *et al*.,(2022), However, report of (Ibrahim *et al*., 2010) was significantly higher in non pregnant than pregnant. Bovine brucellosis is susceptibility increases with sexual maturity and pregnancy (Islam et *al*., 2013b). Due to the influence of placental erythritol sugar that facilitate the pathogenesis of brucellosis (Deselegn and Gangwar, 2011).The variation was according to the prevalence of bruceclla organism in the study area introduction and management practices. After exposure allantoic fluid factor and erythritol stimulate the growth of Brucella organism in pregnant animals.

cattle with a prior history of abortion has AOR=4.8 times more likely showed seropositivity than not abortion AOR=5.7 times more likely showed seropositivity than herds not have history of abortionrespectively. This was agreement with report of AOR 3.3 (Etefa *et al*., 2022) (Geresu *et al*., 2016) and (Deresa *et al*., 2020) reported a statistical association of history of abortion and the presence of bovine brucellosis infection.

Parity number greater than two was (OR= 5.6) times more than monoparity consistent with the findings of (Etefa *et al*., 2022). Animals calved 3–5 times had significantly higher odds of brucellosis seropositivity (Shome *et al*., 2023). In present study the reasons of multiparty more showed seropositivity were due to weak immunity of cows to resist some but reverse of this hormone that support the growth of brucella organism present in older cows.

Cows which have history of placental retention AOR=4.3. times more likely showed seropositivity than not have history of retained placenta. The present study agreement with report of (Islam *et al*., 2013a). Cattle with history of repeat breeding which showed seropositivity is 6.9 times more likely than cattle with out history of repeat breeding. This report agreement with finding of 2.7 AOR (Etefa *et al*., 2022) and (Islam *et al*., 2013a).

Cows test for types of service for mating cows breeding all animal showed seropositivity to bovine brucellosis which use natural mating. This is in agreement with report of (Robi and Gelalcha, 2020). Cows bred by natural service were 3-times (OR=3.11) more likely to face abortion compared to those bred by artificial insemination (AI) (Deresa *et al*., 2020). In contrast to this (Geresu *et al*., 2016) infected bulls are unlikely to transmit brucellosis by natural mating, but if its semen is used for artificial insemination (AI) the chance of spreading the disease is very great. According to (Islam, 2016) as artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus. In contrast to this (Kaltungo *et al*., 2014) bulls that remain fertile and functionally active will shed *Brucella* organisms in the semen during the acute phase of the disease and can there fore help in the spread of the disease.

Bulls may increase the risk of infection as they have frequent contact with other herds during the breeding period (Cárdenas *et al*., 2019b).The variation in report summarize as the chance of mating for breeding with natural bulls were very vast the spreading and the prevalence of bovine brucellosis uncontrollable. The present observation summary the situation need to fowrd to reduce prevalenceof bovine brucellosis is bull screened regularly for brucellosis important. A bull as a semen donor used for Artificial insemination must usually screened is best way to block and reduce brucellosisis.

Comparison between Purchas and born at individual animal level purchase animals (OR = 6.5) showed seropositivity than born and at herds level introduction of purchase cattle in to

herd has 5.3(OR=5.3) than born at home.In line with this result of (Etefa *et al*., 2022), (Musallam *et al*., 2015a). Moreover, (Cárdenas *et al*., 2019b) found that introducing new animals from unknown areas was a significant risk factor for *Brucella* infection in cattle, but the report of (Tesfaye *et al*., 2011) showed high seroprevalence in both purchased and home-bred animals, Out side sources for stock replacement one possible way of introducing the disease (Etefa *et al*., 2022). Awareness creatation for the livestock owners when they purchased cattle from market how they make quarantaine and serological screening needed to prevent diseased animals.

The present study herd sizes, at individual animals level with larger herds being almost five times more likely (OR = 5.4) to be seropositive. In agreement with (Mai *et al*., 2012) the odds of brucellosis seropositivity were 3.5 times greater in large herds than small herds.

. Exposure and maintenance of *Brucella* after an abortion increases with larger herd sizes due to increased contact in shared feeding and watering areas (Getahun *et al*., 2023b). In contrast to this finding (Asakura *et al*., 2018) reported that there was no association between herd size and brucellosis. Brucellosis is present according to area (Matope *et al*., 2010).

The variation in reports across different regions of Ethiopia and other countries may be due to several factors, including agro-ecology since probability stay up to month in cool and wet environment, incubation period is risk factors to large herds and farms due after introduction may showed seropositivity and started to secret organism.

In the present study, all cattle showed seropositivity in present study was which managed under extensive management system. In line with this result of (Etefa *et al*., 2022) in Jimma zone and (Teka *et al*., 2019) the report in south west Shewa, Oromia regional state Ethiopia,. were unable to find bovine brucellosis sero positive reactor in semi intensive. In contrary to the present finding there are higher prevalence in organized dairy farms than in marginal herds (Minda *et al*., 2016). A comprehensive management system increase the contact with infected animals (Asmare *et al.,* 2013). The variation between seropositivity at differrent management system among different site were attributed to free movement of animals, purchase of infected cattle and poor husbandry practices. All bovine this showed seropositivity which used surface water. In line with agreement with report of (Etefa *et al*.,

2022) who reported contamination of water risk factors. The result obtained from this study indicated that water could be predisposing factor to bovine brucellosis.

In herds level cattle kept with a mix of goats, sheep and cattle have a signifcantly higher likelihood of testing positive for Brucella (OR = 4.3) compared to those kept with only cattle. The results of this study align with report of (AOR = 8.3) by (Etefa *et al*., 2022). Brucella *melitensis* has been found in cattle as well as report of (Smits, 2013). This suggests that mixing animal species could have contributed to the spread of the pathogen from small ruminants to cattle (Robi *et al*., 2023). . Contrast to this (Megersa *et al.*, 2012) reported the detection of brucellosis in cattle, sheep and goats have not association. The variation was according seroprevalence in study area.Therefore, separate grazing is highly recommended to prevent bovine brucellosis.

Socio-demographic profile of the respondents indicated that 79% were males and 21% were females. The age category exhibits that the majority of the respondents (92 %) were ages between the 42 and 60 years while 27-41 years of age were 8%. The educational background of the study participant indicated that most of the respondents (83%) primary and (5%) high school attended. Likewise illiterate and 2% degree and above 10 % illiterate. From the result, it can be concluded that the majority of respondents of this study were primary school which may be important to adopt and implement health education particularly zoonotic diseases.

Out of 129 participants, 32 (24.8%) indicated they had heard of brucellosis previously. Our finding is similar report from Pakistan, in which 80% of participants did not know about brucellosis (Arif *et al*., 2019) and the KAP study in Tajikistan, where only 15% had heard of brucellosis (Lindahl *et al*., 2015). The present study on knowledge was higher knowledge than study in Senegal, none of the farmers knew about brucellosis (Kothalawala *et al*., 2018). A study reported from India, 4.8% of farmers knew about brucellosis (Deka *et al*., 2020). Study from Ecuador (0.6% to 30.2%) respondents stated to have knowledge (Ruano and Aguayo, 2017). In present study 29.8% respondents knew the clinical signs of the disease in cattle. This agreement with (Tempia *et al*., 2019) farmers (19.2%) in South Africa was aware about abortions related to brucellosis This finding is better than a study from Sri Lanka, 8.3% of farmers were aware of abortions due to brucellosis (Kothalawala *et al*., 2018) .

The results were found to be less than 48.0% of farmers knew about brucellosis in the Arsi zone, Oromia region, Ethiopia (Tschopp *et al*., 2013). Studies conducted in northern Uganda (Muloki Nabirye *et al.*, 2017) and Kenya (Obonyo and Gufu, 2015) revealed that 63% and 79% of community participants had heard of brucellosis, respectively. The main sources of information in the present study were veterinary services (90.6%; 29/32) how cattle was infected. This finding is in agreement with a study finding from South Africa that the main source was veterinary consultation (Tempia *et al.*, 2019) and (Lindahl *et al.*, 2015).

The main observation showed that 75.2% (97/129) of the respondents were not aware of ozoontic diseases.This finding is higher knowledge than a study report from Pakistan, where 3.0% of the farmers were aware of zoonotic importance (Peck *et al*., 2019). This report in Sri Lanka, where only 2.6% of farmers knew about brucellosis as a zoonotic disease (Hlaing *et al.*, 2024). However, higher knowledge about bovine brucellosis levels from Portugal, 74.7% (Díez and Coelho, 2013) and in Egypt, 96.3% of farmers were aware of transmitted (Holt *et a*l., 2011) .

Among those who knew the transmission, drinking **r**aw milk (12.5%; 4/32), assisting with calving or handling placentas (3.1%; 1/32), and handling an abortion (81%; 26/32) reported as possible means of zoonotic transmission. Raw milk consumption had a significant association with rural-urban consumers, (Deka *et al*., 2020). Similar to the study in Pakistan, 37.3% of the respondents agreed that raw milk consumption could be linked to brucellosis .Slaughtering of infected cattle source of infection for butchers (Madut *et al.*, 2019).

Respondents had a positive attitude towards some questions. (93%; 120/129) stated that they would like to receive more information. Similarly, a study conducted in northern Uganda showed a positive attitude among community participants (Muloki Nabirye *et al.*, 2017) From source 50/129 from public health professionals, 70/129 from veterinary services**.** In contrast, study in Kenya found that 97% requested more information and preferred the local FM radio stations (Obonyo and Gufu, 2015) .

To ensure cattle health, 10% (13/129) of respondents stated that they sought veterinary advice. 12.4% (16/129) bought from people they knew or trusted, zoonotic infections from infected animal materials to humans led to human infection in Tanzania (Schoonman, 2007) .

Our findings reveal that at least three-fourths of the respondents (75.2%) had poor knowledge of brucellosis in cattle with respect to its name, causes, which animals infect, symptoms, mode of transmission, particularly from animals to humans, and methods of prevention. From our findings, most (84.5%) of the respondents consumed raw or soured milk. This practice is corroborated by findings that revealed in Fulani tradition, if milk is heated, cow's udders dry up (Sanhoun *et al.*, 2020) and (Phiri *et al.*, 2021). Consumption of unpasteurized dairy products risk factor for humans brucellosis (Majzobi *et al*., 2022).

Further, the majority (72%) of the respondents demonstrated poor hygiene practices like assessing during animal delivery, removing of abortion with bare hand during husbandry activities that enhance zoonotic infections from infected animal materials to humans (Nyokabi, 2015) .

The results of respondents' knowledge and practices about brucellosis showed that most of the livestock owners' didn't have adequate knowledge and good practices about brucellosis, but all respondents had a positive attitude. The potential risk factors and association with bovine brucellosis seropositivity in present study were summarized as lack of adequate knowledge and good practices about brucellosis, management-related risk factors resulted to exposure their cattle to contaminated area.

The present study is one of many cases of the condition that have been studied in Ethiopia. Surveillance, transmission prevention and culling are effective control techniques for bovine brucellosis (Durrani *et al*., 2020). Even though the study's goal was to select animals at random, it's probable that inadequate sample frames contributed to bias in the results. Farmers may describe desirable practices that they may not always follow, which could exaggerate the positive practices. Enhancing livestock owners' toward disease prevention and animal health could lead to more effective control efforts.

The knowledge, attitude and practice survey plays a vital role in developening nations with limited resources, evaluating livestock owners' understanding and preparedness against such livestock diseases (Musallam *et al*., 2015b). Inter-disciplinary approach is an effective way to eradicate this disease (Zeng *et al.*, 2019).Therefore, increase public awareness about the transmission of brucellosis and further research should be conducted on brucellosis in high-risk groups.

6. CONCLUSION AND RECOMMENDATIONS

From this study, it can be concluded that bovine brucellosis was found with slightly higher seroprevalence in the study area that coused economic significance was high. Risk factors such as status of pregnancy, abortion, and retained placenta, repeat breeding, and herd size, source of replacement livestock, feeding style, and management system were significantly associated with the occurrence of the disease. In the present study, bovines that showed seropositivity were adults, local breeds, only animals managed at under extensive management system and most of them females which have a history of multiparous. Only a very small number of livestock owners' knew about the bovine brucellosis disease. Even though several respondents had a positive attitude score on bovine brucellosis, due to a poor knowledge score, most of the participants in this study were involved in malpractices such as the handling of fetal membranes with the bare hand and throwing away aborted materials and fetal membranes into the environment, which worsened contamination of the environment and facilitated maintenance as well as transmission of the pathogen. Therefore, based on this conclusion, the following recommendations are pinpointed:

- ✓ Isolation of pregnant animals from herds reduces the risk of abortion in herds; it may reduce the risk of exposure and infection; if infected and aborted outside of herds, there may be a low risk of transmission to other animals.
- ✓ Public health education campaigns, such as biosafety measures and zoonotic transmission of bovine brucellosis, need to be undertaken by the government and other responsible bodies.
- ✓ Continuous surveillance to detect the presence of the infection on dairy farms should be designed and implemented to save public health importance.
- ✓ Collaborative multisectoral approach designed to tackle the transmission of zoonotic diseases between humans and animals by bringing together or require the coordinated and combined efforts of physicians, veterinarians, epidemiologists, ,environment,forest, agriculture,climate change , in generally health sectors and other experts to understand the ecology of each emerging zoonotic disease.

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8. APPENDICES

Appendices 1: Data collection format used to collect information on cattle brucellosis

Date---------

Region ------Zone-------District ----- PA------Village--------GPs; Long-----Lat___Alt----------

No	Owners name	species	breed	sex	age	parity	HA	HP	RP	HRB	Sample code	Animal origin		C-ELISA result
												born	bought	
1														
2														
3														
4														
5														
6														
7														

HA=history of abortion,HP=history ofpregnant ,RP=retained placenta ,HRB=history of repeat breeding, RP reained placenta (Yes/No); History of abortion (Yes /No)

Appendices 2. Socio demographic characterstic of respondents

1) Name of respondents ---------- IDN----------

2) Address of the owner District-- -; Village 3) Gender; 1) Female 2) Male

Animal code

Date

4) Age (years);

5) Marrital status; 1) single 2) Married 3) Divorced 4) Widowed

6) Education level; 1) Primary school 2) Secondaryschool 3) Vocational school 4) Diploma

5) Degree F) Illiterate

Appendices 3: data collection formate for the study of risk factor of Bovine brucellosIs

I. General information of the animals

1. Breed-----Sex-----Age-----herd size----T/A/S per herd--

2. Parity 1) 1-2 2)3-4 3) >5 4)none

3. Reporductive category 0) bull 1) Heifers 2)Cows

4. Status of pregnancy 0) Not pregnant 1)pregnant

5. History of abortion 0) Not pregnant 1) Yes

6 History of retained fetal placenta 0) Not pregnant 1) Yes

7. History of repeat breeding 0) No 1) Yes

8. Farming system 0) Intensive 1) Extensive 2) semi-intensive

II. Information on farm management

1. Is there frequent contact between your animals with other herds? 0) no 1) yes

2. How is the feeding style of your cattle? 0) grazing separately 1) mixed with other livestock 2) both

3. From where do you get cattle to introduce for replacement of stock?

? 0) Market 1) Raise own replacement 2) Both

4. For breeding cattle what type of service you have? 0) AI 1) Natural service or Bull 2) Both

5) What types of watering system you use? 0) Tap 1) Underground water 2) surface water

6) Have you aborted bovine encountered with in your herd of animals? A) Yes B) NO C)I don't know

III .knowledge questions

1 .Do you rearing cattle? 0) no 1) yes

2 .Do you know a disease called bovine brucellosis? 0) No 1) yes

3 .If yes from where did you hear about it? 0) vet.service 1) community gathering and or talk 2) Rado and or television3) Meeting

4. If yes, do animals infected with bovine brucellosis? 0) No 1) Yes

5 .If yes, which groups of animals? 0) Cattle 1) Shoats 2) All animals 3) I don't know.

6. If yes, is bovine brucellosis transmitting between cattle? 0) No 1) Yes 2) I don't know

7. If yes, how bovine brucellosis transmit between cattle? 0)Abortion 1)shared grazing place 2)shared pens 3)placenta from live births 4) all 5)I don't know

8. What are the symptom do you know in cattle? 0) Abortion 1) born weak calves 2) bull infertility 3) retained fetal placenta 4) all 5) I don't know

9. Do you know that bovine brucellosis transmit from animas to human? 0) No 1)

10. If yes, by what mechanisms? 0)drinking of raw milk 1) assissing of calving and or handling placenta 2) handling of abortion 3) slaughtering infected animals 4) all 5) I don't know

11. How bovine brucellosis a preventable disease? 0) Avoiding drinking raw milk 1)avoiding careless handling of aborted fetus and or retained fetal placenta 2)avoiding consumption of undercooked food 3) all F) I don't know

IV. Attitudes questions

1. Do you believe that you or your family members working most with the cows exposed to the Brucella infection are at high risk of infection? 0) No 1) Yes

2. Do you need to know more information about the disease? 0) No 1) Yes

3. If yes, from which source do you want the information? 0)public health professionals 1)family and friends 2) meeting in the village 3)Radio/television 4) veterinary service s 5) all

4. How health is ensured when receiving new cattle? 0) seek veterinary advice 1) Buy from people knowns and/or trusted 2) Rely on own experience 3) I don't care

5. What do you think about the general hygiene of the house of herd? 0) very good 1) good 2) satisfactory 3) poor

V. Practice question

1. Do you drink raw milk and its products? 0) No 1) Yes

2. Do you eat raw meat? 0) No 1) Yes

3. Do you separate cows during parturition? 0) No 1) yes

4. How do you handle the aborted fetus and placental membranes? 0) bare hands 1) using glove 2) I don't care

5. Who assist your cattle during delivery? 0) veterinarian 1) shepherds 2) household members 3) I don't care

6. Farm practices regarding the disposal of aborted fetuses. 0) Bury in ground 1) Give to dog 2) Throw a ways d 3) I don't care

7. What do you do if placenta membrane found? 0) Throw away or dump 1) Burn 2) Bury in ground 3) Give to dog 4) Nothing

8. Do mixed diffirent specieslike shoatwith cattle during grazing on one farming place? 0) No 1) yes

9 .Do you use AI service for cattle breeding 0) yes 1) No

Appendices 4: proceture technueque cELISA

Proceture

Initially 1.All regeants was equilibrated to room temperature 18-25^{0}c (64-77 0F) before use as directed by the manufacture.

2.1. First add samples 45microlitre of sample dilution buffer was added into each well that used for serum samples, serum controls and conjugate controls

2.2. Then, add 5microlitre of positive, weakpostive and negative serum controls was added into each of the appropriate wells respectively .for confirmation purposes it is recommended to run the control sera in dubplicates.

2.3. Next, 5 microlitre of sample dilution buffer was added into two appropriate wells designated as conjugate control, Cc).

2.4. After that, 5microlitrel of the test sample was added into each of the appropriate wells; the sample was run in duplicates for confirmation purpose.

3. Then, 50 microlitrel of mab solution was added into all wells used for controls and samples; the time difference between controls or sample and mAB-solution addition was 10 minutes.

4. Then, the plate was sealed and the regeant was mixed thoroughly for five minutes

5. The plate was incubated at room temperature (18-25 ^{0}c or64-77 ^{0}F) for 30 minutes.

6. After 30 minutes,the plate was rinsed 4 times using PBS Tween Buffer solution and allowed to dry by using towel;

7. Then, 100 microlitrel of conjugate solution was added into each well and the plate was sealed and incubated at room temperature for 30 minutes;

8. Step number 6 was repeated

9. After that, 100microlitre of substrate solution was added to all wells and incubated for 10minutes at room temperature;

10. Then, 50 microlitre of stop solution was added into each well to stop the reaction and mixed thoroughly the stop solution was added in the same order as the substrat solution in step 9;

11 Finaly, the result was decided by measuring the optical density (OD) of the controls and samples at 450nm in a microplate photometer (ELISA Reade). Measured the OD with in 15 minute after the addition of stop solution to prevent fluctuationin OD vaues.

Calculation; 1) The mean OD values of controls and samples are calculated automatically by using computer system and then percent of Inhibtion (PI) values are calculated for controls and as well as samples by using the following formula ;

$$PI = \frac{100 - (OD\ samples\ or\ control \times 100)}{OD\ conjugate\ control}$$

Interpretation

The results of the serology are determined as positive if PI>30 but negative if pi<30

Appendices 5: Age determinationon cattle based on teeth eruption

No	Teeth	Ages
1	2erupts	1.5-2years
2	12erupts	2-2.5years
3	13erupts	3years
4	C erupts	3.5-4years
5	All incisors are wear	5years
6	11 is level and the neck has emerged from the gum	6 years
7	12 is level and the neck is visible	7 years
8	13 is level the neck is visible	8years
9	C is level and the neck is visible	9 years
10	The teeth that have not fallen out arereduced to small round pegs	15 years

Source; pace and wakeman, 2003

Appendices 6: Pictures during questionnaire survey on analysis KAP or risk factors

A= During KAP quetionarries enterview the respondents

B= Livestockowners' and cattle C and D= during blood sampletaken

Appendices 7: Pictures during laboratory procedure analyses

A= During serum sapled processing in laboratory.

B= Chemical used for serum sampledprocessing in laboratory

C= Resulted serum that measured the opitical density at45nm in a microplatephotometer