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Seroprevalence, Assessment of Knowledge, Attitudes, and Practices (KAP), and Public Health Implications of Bovine Brucellosis in West Hararghe Zone, Oromia Regional State, Eastern Ethiopia

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Abstract

Bovine brucellosis is a bacterial disease that affects both cattle and humans. This study aimed to estimate the seroprevalence of bovine brucellosis in four districts of West Hararghe zone, Eastern Ethiopia, using mRBPT and I-ELISA tests. The study was conducted from July 2024 to February 2025 G.C. using a cross-sectional design and random sampling. A total of 384 cattle of local breeds were sampled from two extensive and two semi-intensive management systems. The overall individual animal seroprevalence was 3.9% by mRBPT and 3.1% by I-ELISA. The seroprevalence was higher in the extensive (2.9%) than in the semi-intensive (0.3%) management system. Larger herd sizes, older age groups, females, lowland agroecology, and pastoral production systems were associated with higher seroprevalence. A structured questionnaire was used to interview 100 cattle owners to assess their (KAP) regarding bovine brucellosis and its public health importance. The results showed that 80% of the respondents had no information about brucellosis, 73% had risk practices, and 83% had negative attitudes, which could facilitate the transmission of brucellosis between cattle and humans. Our findings suggest that bovine brucellosis is prevalent in the study area and poses a significant zoonotic risk. We recommend implementing control measures to prevent the spread of the disease and to raise awareness among cattle owners and consumers.

Keywords: attitude; brucellosis; knowledge; practices; seroprevalence

Introduction

A Sub-Saharan African country, Ethiopia, faces a high risk of zoonotic diseases and poverty, which are closely linked. This is because of the potential for zoonotic disease transmission and the emergence and re-emergence of pandemic threats (Epiz, 2019). The country has 70,291,776 million cattle in total, and 22,598,088 million of them are dairy animals, making up 32.15% of the total cattle population. About 80% of Ethiopians rely on agriculture and have close ties with their livestock. As a result, the country is susceptible to the spread of zoonotic diseases among these populations (Gumi et al., 2013).

Brucellosis is the most important neglected zoonotic disease in Ethiopia, with significant public and economic impacts (Pieracci et al., 2016). Ethiopia's cattle population has a great opportunity to access live animal and red meat export markets in the Middle East, Gulf States and some African countries. However, production is hindered by challenges such as lack of feed and water, poor husbandry management and diseases including brucellosis, which limit the export market (Geletu et al., 2021). Brucellosis, especially bovine brucellosis, has caused serious economic problems in cattle in countries like

Ethiopia, due to indirect losses (reduced fertility, milk production and sale value of infected cows, trade restrictions of live animals and their products; disruption of local markets, and obstacles to free animal movement) and direct losses (abortion, neonatal death, replacement costs, treatment costs, emergency slaughtering of infected animals and stillbirths) (Díaz, 2013).

The genus *Brucella* is Gram-negative, facultative intracellular, coccobacillus, non-spore-forming, and non-motile bacteria comprised of different species affecting preferred host species. Currently, ten species including the better-known six classical species comprised of *B. abortus*, *B. melitensis*, and *B. neotomae* are known. In recent times, other new species of the genus including *B. ceti*, *B. pinnipedialis*, *B. microti*, and *B. inopinata* which affect different species of animals are also identified (Godfroid et al., 2011). Bovine brucellosis, mainly caused by *B. abortus* and sometimes by *B. melitensis* and *B. suis*, is a serious infectious disease that affects cattle productivity (especially in the dairy sector) and human health in Ethiopia (Nuraddis et al., 2010, Reta Duguma & Damena 2016). The disease is endemic and poses a major public health threat in the country

due to the high prevalence of these factors, the close contact between humans and animals, and the food consumption habits of the people. Moreover, many countries have not implemented control or eradication programs for brucellosis (Barrio et al., 2009). Brucellosis is one of the most widespread and economically significant diseases in tropical and sub-tropical regions. The disease causes direct losses of meat (due to abortion, infertility and weight loss) in infected cattle herds of about 15%, and of milk (due to reduced milk production) of about 20% (Zhang et al. 2018). Among the infectious diseases, *Brucella* infection is widely prevalent and causes extensive economic losses, and brucellosis is one of the most serious zoonotic diseases in Ethiopia (Hagos et al., 2016).

The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water, and after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitute a very important source of infection (Acha and Szyfres, 2001). Sources of infection for the transmission of the bovine brucellosis are aborted fetuses, the fetal membranes after birth, and vaginal discharges and milk from infected animals (Moti et al., 2013, Acha and Szyfres, 2001). The same organism also causes undulant fever in man from drinking raw or un-pasteurized infected milk or milk products or from exposure of farmers, packing house workers, veterinarians and others to infected discharges or tissues (Dirar et al., 2016). Brucellosis is a disease that affects both animals and humans. It causes abortion, metritis, infertility, and reduced milk production in cows, and orchitis and epididymitis in bulls (Tucho and Jimma, 2017). In humans, it causes fever, depression, weight loss, joint pains, headache, and anorexia. Brucellosis has a significant economic and public health impact, especially in developing countries where dairy production is important (Melese et al., 2016). The disease also restricts animal movement and trade, and increases the healthcare and non-healthcare costs for the infected people (Khurana et al., 2021).

Statement of the Problem

Bovine brucellosis is a zoonotic bacterial disease that affects cattle and humans. It is caused by *Brucella abortus*, a gram-negative intracellular bacterium that can infect various domestic and wild animals. The disease is one of the most important livestock diseases in the world, affecting animal health, productivity and

trade. It is also a serious public health problem, especially in developing countries where people have close contact with animals and consume unpasteurized dairy products (Poester et al., 2013). Bovine brucellosis causes abortion, infertility, reduced milk production and economic losses in cattle. In humans, it causes undulant fever, arthritis, endocarditis and other chronic complications. The disease is transmitted through direct contact with infected animals or their products, such as milk, meat and cheese (Pal et al., 2017).

The disease is endemic in many developing countries, including Ethiopia, where livestock production is a major source of income and food security for the rural population. However, the epidemiology and public health impact of bovine brucellosis in Ethiopia are poorly understood. There is a lack of reliable data on the seroprevalence, risk factors, knowledge, attitude and practice (KAP) of the disease among cattle owners and consumers. This hampers the implementation of effective control measures and poses a threat to human and animal health (Berhanu et al., 2020). The seroprevalence of bovine Brucellosis is a crucial public health issue, especially for those who own livestock or consume animal products, because it can help plan effective control measures. However, in the study area, no previous research has been done on the seroprevalence and public health impact of bovine brucellosis. Therefore, the farmers lack reliable information on this disease and consider abortion as a major problem. This motivated us to investigate the situation and public health implication of bovine brucellosis.

The general objective of this study is to estimate the seroprevalence, assess the public health implication and KAP of the community on bovine brucellosis in four selected districts (Chiro, Daro labu, Mieso and Burka dhintu) in West Hararghe zone of Oromia regional state, eastern Ethiopia. These districts are characterized by mixed crop-livestock farming system and high human and cattle population density. The study will provide valuable information on the burden and awareness of bovine brucellosis in the study area and will contribute to the development of appropriate control strategies.

General Objective

- To estimate the seroprevalence, assess the public health implication and KAP of the community on bovine brucellosis in four selected districts (Chiro, Daro labu, Mieso and Burka dhintu) in West

Hararghe zone of Oromia regional state, eastern Ethiopia.

The Specific Objectives of the Study are

- To estimate the seroprevalence of bovine brucellosis in the cattle population in the study area using Rose Bengal Plate Test (RBPT) and Enzyme Linked Immunosorbent Assay (ELISA).
- To assess the public health implication of the disease in the study area.
- To understand the knowledge (awareness), attitude and practice of the community towards zoonotic bovine brucellosis in the study area using a structured questionnaire.

Literature Review

Brucellosis is a disease that affects both humans and animals. It is classified as one of the world's leading neglected zoonotic diseases by the World Health Organization (WHO). The disease is known to severely impact livestock productivity and human health worldwide, with low-income countries being particularly affected (Franc et al., 2018). Bovine brucellosis is of major economic importance in most countries of the world. It affects approximately 5% of the livestock population worldwide and continues to increase in distribution (Khurana et al., 2021). Bovine brucellosis is a bacterial disease that causes abortions and infertility in cattle. It is also a zoonotic disease that can infect humans through contact with infected animals or consumption of unpasteurized dairy products. The disease was first recognized in the Mediterranean region, where it affected sheep and goats. The causative agent, *Brucella melitensis*, was isolated by David Bruce in 1887 (Wongtavatchai et al., 2004). Later, another species of *Brucella*, *Brucella abortus*, was identified as the cause of contagious abortion in cattle by Bernard Bang in 1897. The genus *Brucella* was named after Bruce by Karl Meyer in 1920 (Hall, 2020).

Brucella abortus is the causative organism for bovine brucellosis and at least nine biotypes have been recognized including a number of strain variants (Radostits et al., 2000). *Brucella abortus* is mainly infective for cattle. Cattle can also become infected by *B. suis* and *B. Melitensis* when they share pasture or facilities with infected pigs, goats, or sheep. The infections in cattle caused by heterologous species of *Brucella* are usually more transient than that caused by *B. Abortus* (Zriba et al., 2019).

Epidemiology

Etiology: *Brucella* spp. is small (0.5–0.7 by 0.6–1.5 µm), non-motile, non-spore-forming, slow growing, facultative intracellular Gram-negative coccobacilli belonging to the Brucellaceae family along with the *Mycoplana* and *Ochrobactrum* spp (Di Bonaventura et al., 2021, Jiao et al., 2021). Brucellae are capable of infecting several animal species and are currently classified into 12 species. Four of these species are responsible for almost all human infections: *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*. Among these species, *B. melitensis* is considered the most virulent (Whatmore et al., 2016).

Occurrence and Prevalence of Infection: Brucellosis has a worldwide distribution and is mainly a problem in developing countries such as Ethiopia (Dereje, 2022). In Jordan, the highest prevalence of brucellosis was found in cattle and sheep (Hotez et al., 2012). The prevalence of brucellosis in cattle and buffaloes in the north-western state of India called Juban was found to be 20.67% and 16.41%, respectively. The overall apparent prevalence of brucellosis was noted to be 18.26% (Aulakh et al., 2008). In Ethiopia, most research done on brucellosis has been focused on intensive dairy cattle herds in urban and peri-urban areas (Sima et al., 2021). A large number of articles have been published reporting individual seroprevalence ranging from 1.1% to 22.6% in intensive management systems and 0.1–15.2% in extensive management system (Hailu et al., 2011). The overall seroprevalence of bovine brucellosis in pastoral and agro pastoral regions of East Showa Zone, Oromia Regional State, was 11.2% by the Rose Bengal Plate Test (RBPT) (Mangen et al., 2002). In a study of bovine brucellosis in cattle under traditional production system in North-West Ethiopia Benishangul-gumuz, 14 out of 1,152 cattle screened for *B abortus* antibodies tested positive by RBPT. Of these, 11 animals (79%) were confirmed positive by complement fixation test (CFT), giving an apparent seroprevalence of 1.0% in the study area (Adugna et al., 2013, Sintayehu et al., 2015).

Source of Infection and Mode of Transmission in Animals: Brucellosis is typically transmitted to other cattle by direct or indirect interaction with diseased cattle or their discharges. The spread of brucellosis in cattle occurs through the ingestion of contaminated feed and drinking water contaminated by the bacteria that are present in massive amounts in birth products and uterine discharge (Pal et al., 2017). In cattle, the main route of infection is through licking the fetuses or newborn calves, which are highly contaminated

with bacteria (Donev, 2010). Another way of transmission is by giving pooled colostrum to calves. Sexual contact is not a common cause of infection in cattle, but artificial insemination can introduce the bacteria from infected to healthy animals (Robinson and Production, 2003). In humans, the most frequent way of getting infected is by consuming unpasteurized milk or dairy products. Direct contact with the fluids or tissues of aborted fetuses from infected cattle can also infect humans through the mucosa or wounds (Quinn et al., 2011). People who work with cattle or their products are at high risk of brucellosis. This includes abattoir, farm, and laboratory workers, as well as veterinarians (Memish and Mah, 2001). It is rare for humans to transmit the infection to other humans. However, some cases have been reported due to blood transfusion, bone marrow transplantation and sexual contact (Akçakuş et al., 2005).

Clinical Signs in Animals: In cattle, *Brucella abortus* causes abortions, stillbirths and weak calves. (Dougherty et al., 2013). The placenta may be retained and lactation may be decreased. Epididymitis, seminal vesiculitis, orchitis and testicular abscesses are sometimes seen in bulls. Infertility occurs occasionally in both sexes, due to metritis or orchitis/epididymitis. Hygromas, particularly on the leg joints, are a common symptom in some tropical countries. Arthritis can develop after long-term infections. Systemic signs do not usually occur in uncomplicated infections, and deaths are rare except in the fetus or newborn (Stear 2005). Infections in non-pregnant females are usually asymptomatic, but pregnant adult females infected with *Brucella abortus* develop placentitis, which normally causes abortion between the fifth and ninth month of pregnancy (Saxena et al., 2015). Even in the absence of abortion, there is heavy shedding of bacteria through the placenta, fetal fluids and vaginal exudates. The mammary gland and regional lymph nodes can also be infected and bacteria can be excreted in milk (Pal et al., 2017). The incubation period varies between 14 and 120 days (Costard et al., 2009).

Clinical Signs in Humans: The symptoms of brucellosis are nonspecific. The majority of patients complain of fever, sweats, malaise, anorexia, headache, arthralgia, and back-ache (Doganay and Aygen, 2003). Brucellosis is a systemic disease in which any organ or body system can be involved. The incubation period varies between 1 and 5 weeks, and

Brucella infection may be asymptomatic or symptomatic (Eshetu Yimer et al., 2008). The onset of symptoms is acute or insidious. According to the length and severity of symptoms, the disease is arbitrarily classified as acute (less than 8 weeks), sub-acute (from 8 to 52 weeks), or chronic (more than 1 year). Any organ involvement is often referred to as localized disease. It can be seen as a complication of acute brucellosis or may be the only manifestation of chronic brucellosis (Govindasamy, 2020).

Pathogenesis: *Brucella* species are facultative intracellular pathogens and establish infection by invading macrophages and evading macrophage-induced host protection mechanisms (Pal et al., 2017). *Brucella* species are capable of invading macrophages and epithelial cells of the host, allowing infection through mucosal surfaces. Once *Brucella* species have invaded, they are capable of surviving intracellularly within phagocytic or non-phagocytic host cells (Neta et al., 2010). *Brucella* has the ability to interfere with intracellular trafficking and prevent fusion of the *Brucella*-containing vacuole with lysosome markers. This directs the vacuole towards a compartment that has rough endoplasmic reticulum which is highly permissive to intracellular replication of *Brucella* (Pizarro-Cerdá et al., 2000). These characteristics contribute to clinical signs and therapeutic considerations, including the difficulty in both diagnosis and treatment (Hamid 2012). Following exposure in humans, the organisms travel along the lymphatic pathways; focal disease is most commonly identified in the reticulo-endothelial tissues such as the liver and spleen. In chronic infections, organisms typically localize in joints, especially large joints such as the sacroiliac or lumbar vertebral joints. Pulmonary disease is a less common form of brucellosis (Glynn and Lynn, 2008).

Risk Factors for Brucellosis

Host Risk Factors: Brucellosis is a disease that can infect a variety of domestic and wild animals and humans. The susceptibility of animals to *Brucella* infection is influenced by age, breed, and pregnancy status (Gusi et al., 2019). Sexually mature animals are much more susceptible to infection than younger animals. Herd size and animal density are directly related to the prevalence of the disease and difficulty in controlling infection in the population (Islam et al. 2013). Sexually mature pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex. Susceptibility

increases as the stage of gestation increases (Radostits et al., 2006).

The predilection sites for *Brucella* are the reproductive tracts of males and females, especially the pregnant uterus. Erythritol is present in the placenta and male genital tract of cattle, sheep, goats, and pigs but not in humans. Allatoic factors stimulate the growth of most *Brucella* (Melese, 2016).

Agent Risk Factors: *Brucella abortus* is a facultative intracellular organism that can multiply and survive within the host phagosomes. Some of the organisms are phagocytized by polymorph nuclear leucocytes in which they survive and multiply. The bacterium can survive within macrophages because it has the ability to survive phagolysosome (Kang'ethe et al., 2000). The bacterium has an unconventional non-endotoxin lipopolysaccharide that confers resistance to antimicrobial attacks and modulates the host immune response. These properties make lipopolysaccharide an important virulence factor for *Brucella* survival and replication in the host (Radostits et al., 2006). Naturally infected animals and those vaccinated as adults with strain 19 remain positive to the serum and other agglutination tests for long periods. The antibody response to *Brucella* consists of an early IgA and IgM is a type response, the timing of which depends on the route of exposure, the dose of bacteria, and the health status of the animal. The IgM response is followed shortly by production of IgG1 antibody and later by IgG2 (Nielsen and Yu, 2010).

Occupational Risk Factors: Laboratory workers handling *Brucella* cultures are at high risk of acquiring brucellosis through accidents, aerosolization and/or inadequate laboratory procedures. In addition to this, abattoir workers, farmers and veterinarians are at high risk of acquiring the infection (Coulibaly and Yameogo, 2000). Acquiring infection through direct contact is a potential threat to occupational groups such as farmers, veterinarians, butchers, laboratory workers, milkers and inseminators. Handling aborted materials or attending retained placenta or dystocia without protective gear is a common practice to most field veterinary assistants, abattoir workers and in many rural pastoral settings (Kohl & Ascoli 2017). This may suggest that animal health workers and rural communities are also at great risk of contracting the disease if the disease is present in domestic animals (Luelsegged et al., 2018).

Management Risk Factors: The unregulated movement of cattle from infected herds or areas to brucellosis-free herds or areas is the major cause of

breakdowns in brucellosis eradication programs. Once the herds are infected, the time required to become free of brucellosis is increased by large herd size, by active abortion, and by loose housing (Muma et al., 2007). The spread of the disease from one herd to the other and from one area to another is almost always due to the movement of an infected animal from infected herd in to a non-infected susceptible herd (McGivern et al., 2003). A case-control study of brucellosis in Canada indicates that, herds located close to other infected herds and those herds whose owners made frequent purchase of cattle had an increased risk of acquiring brucellosis (Radostits et al., 2006).

Diagnosis: The disease can be diagnosed using various methods that involve bacteria, serum or DNA. Some of the methods are: detecting bacteria in samples, amplifying DNA with PCR, and measuring serum reactions with milk ring test, complement fixation test, rose bengal plate test and ELISA. (Padilla Poester et al., 2010, Pinto Ferreira et al., 2022).

Serological Tests

Rose Bengal Pate Test (RBPT): The Rose Bengal test (RBT) is a screening test for the diagnosis of brucellosis in animals and humans (Corbel, 2006). The Rose Bengal plate test (RBPT) is a simple and rapid test for the diagnosis of bovine brucellosis. It is based on the agglutination of stained *Brucella abortus* antigen by serum antibodies. The test can be performed in the field or in the laboratory, and does not require any special equipment or reagents (Roth et al., 2003). The RBPT has a high sensitivity and specificity, and can detect both acute and chronic infections. However, the test may also give false positive results due to cross-reactions with other bacteria or vaccination. Therefore, the RBPT should be used as a screening test, and positive results should be confirmed by other methods such as ELISA or culture (Nielsen et al.; 2004; Radostits et al.; 2007 and OIE., 2019).

Indirect Enzyme-Linked Immunosorbent Assay (i-ELISA): Bovine brucellosis is a zoonotic disease caused by *Brucella abortus*, which affects cattle and humans (Godfroid et al., 2011). The diagnosis of this infection is important for animal and public health, as well as for trade and epidemiological purposes (OIE, 2016). Among the various serological tests available, the indirect enzyme-linked immunosorbent assay (i-ELISA) is widely used because of its high sensitivity and specificity, as well as its ability to process a large number of samples in a short time

(Nielsen and Yu, 2010). In this review, we describe the principles, procedures, advantages and limitations of the i-ELISA for the detection of antibodies against *B. abortus* in bovine serum samples. We also discuss the factors that can influence the performance and interpretation of the test, such as the antigen preparation, the quality of the reagents, the cut-off value, the cross-reactivity with other bacteria and the validation criteria. Finally, we provide some recommendations for the standardization and optimization of the i-ELISA for bovine brucellosis diagnosis. Indirect enzyme-linked immunosorbent assay (i-ELISA) is a widely used serological method for the diagnosis of bovine brucellosis, a zoonotic disease caused by *Brucella abortus* (Godfroid et al., 2004). This review aims to provide an overview of the principles, applications, advantages and limitations of i-ELISA for bovine brucellosis. The i-ELISA is based on the detection of antibodies against the smooth lipopolysaccharide (S-LPS) antigen of *B. abortus* in serum samples (Nielsen and Yu, 2010). The S-LPS antigen is coated on microtiter plates and incubated with serum samples. After washing, a secondary antibody conjugated with an enzyme is added to bind the primary antibody. The enzyme catalyzes a colorimetric reaction that indicates the presence or absence of specific antibodies. The i-ELISA has several advantages over other serological tests, such as high sensitivity and specificity, ability to test large numbers of samples, automation and standardization (OIE, 2016). However, i-ELISA also has some limitations, such as cross-reactivity with other bacteria, interference by maternal antibodies, variability in antigen quality and assay performance, and need for specialized equipment and reagents (Gall et al., 2010). Therefore, i-ELISA should be used in combination with other tests and epidemiological data to confirm the diagnosis of bovine brucellosis (McDermott and Arimi, 2002).

Polymerase Chain Reaction: One way to identify *Brucella* DNA in pure cultures and in various clinical specimens, such as blood, urine, tissues, and fluids, is to use polymerase chain reaction (PCR) assays (Colmenero et al., 2010). However, this method faces some difficulties because the clinical samples have very low levels of bacteria and contain substances that can interfere with the PCR reaction. Therefore, it is important to use sample preparation methods that can reduce the interference and enrich the bacterial DNA template (Queipo-Ortuño et al., 2008).

Treatment: The best antibiotic treatment for brucellosis remains controversial despite many studies in the last 15 years. The World Health Organization advises to use rifampicin 600 to 900 mg and doxycycline 200 mg daily for at least 6 weeks for adults with acute brucellosis, but some argue that the old combination of streptomycin by injection and an oral tetracycline causes less relapses (Ariza et al., 2007).

Prevention and Control

To control brucellosis, different methods can be applied depending on various factors. One method is to test the animals and either isolate or slaughter those that are positive. This method is more effective when the infection rate is very low in the herd or flock. It also depends on the availability of funds, regulations and the willingness of the owners to cooperate. Sometimes, compensation is needed. Vaccination can reduce the risk of keeping positive animals, but this should be a last option. Testing and slaughtering is not very useful for sheep and goats, because the tests are not very accurate (Whittington et al., 2019). Another method is to improve hygiene and prevent contact between infected and healthy animals, or their fluids and tissues. This is a basic principle of disease control. However, the success of this method also depends on the type of farming, the level of awareness of the owners, the trade practices, the frequency of symptoms, and the feasibility of separating animals during birth (Lulseged et al., 2018). A third method is to restrict the movement of animals and ensure their identification by brand, tattoo or ear tag. Animals from infected areas should not be sold or moved to other areas without authorization. Likewise, animals from clean areas should only come from areas that are free of brucellosis, that have a history of being disease-free and that have tested negative recently (Mantur and Mangalgi, 2004).

Brucellosis is a disease that affects both animals and humans, and it is transmitted mainly by contact with infected animals or consuming their unpasteurized products. In Ethiopia, where human brucellosis is linked to exposure to infected animals or their products, there is no national or regional program to prevent and control the disease. This is due to the lack of resources and facilities, as well as the inconsistent and sometimes low prevalence data that may undermine the importance of the disease. However, it is urgent to determine the geographical scope of the problem and to allocate funds and resources to implement prevention and control strategies in

Ethiopia and other countries with similar economic situations (Moti et al., 2013). The risk factors of brucellosis may differ from one country or region to another, but most of them are similar. The main route of infection from animals to humans is the consumption of raw milk and dairy products, besides direct contact with infected animals and their fluids (Efa et al. 2012). The most effective way to control this widespread infection is through the One Health approach, which integrates human health, animal health, and environmental health (Bamaiyi, 2016).

Therefore, it is essential to provide educational information about disease prevention to areas where *Brucella* is endemic, in order to control the disease (Asiimwe et al., 2015). Moreover, since infected animals and their products are the main source of human infections, controlling brucellosis in livestock and establishing a functional veterinary infrastructure is a crucial step to reduce the disease in humans (Seleem et al., 2010).

Public Health Significance of Brucellosis

Brucellosis is a serious zoonotic disease caused by *Brucella abortus*, which can infect humans through contact with infected cattle or their tissues. It is also one of the main laboratory infections that can be easily acquired. Another source of infection is consuming raw milk or dairy products (Wubishet, 2018). There are nine known *Brucella* species, and five of them can infect humans. *B. melitensis*, *B. abortus* and *B. canis* are the most pathogenic and invasive species for humans. *B. ceti*, a marine *Brucella*, has also been reported to cause zoonotic infections. *B. melitensis* causes the most severe form of human brucellosis, followed by *B. suis*, *B. abortus* and *B. canis* in descending order (Torregrosa et al., 2021). They are considered as potential bio-weapons by the US disease control and prevention program, because they are highly infectious and can be spread through aerosols. Moreover, brucellosis is hard to detect because its initial symptoms are similar to those of influenza (Chain et al., 2005). Brucellosis affects half a million people worldwide every year. The infection rate in humans is related to the infection rate in animal reservoirs (Scholz et al., 2010).

Humans can get infected by *Brucella* through ingestion, inhalation or contact with contaminated materials. The importance of each factor depends on the epidemiological area, animal reservoirs and occupational groups at risk. In countries where milk and dairy products are always pasteurized, brucellosis mainly affects people who work with animals and

animal products (Al Dahouk et al., 2007). The prevalence of *Brucella* infection in humans is linked to the prevalence in nearby cattle (Mekonnen et al., 2011). Brucellosis is a relatively prevalent infection in humans and animals in developing countries, and it causes several cases every year (Corbel, 2006). People with brucellosis may experience fever, sweat, loss of appetite, fatigue, weight loss, depression, headache, and joint pain. This infection can be mistaken for other illnesses such as malaria and influenza (Dean et al, 2012).

Status of Bovine Brucellosis in Ethiopia

Bovine brucellosis is a widespread and endemic disease in Ethiopia, affecting urban, peri-urban, highland and lowland areas with various types of animal farming, including large and small-scale farms and ranches. The disease causes significant economic losses and poses a serious threat to public health (Megersa et al. 2012). The occurrence and prevalence of brucellosis in Ethiopia differ by location and time, due to variations in animal production and management systems, multiple livestock species per holding, community living standards, awareness levels as well as agroecological conditions. These factors also contribute to the persistence and transmission of risk factors for bovine brucellosis (Bulcha et al., 2020). The disease has major implications for the health and economy of rural communities. Therefore, understanding the disease situation in traditional livestock husbandry practice is crucial for reducing its economic and public health impacts (Chachra et al., 2009).

Seroprevalence of Bovine Brucellosis

According to different research groups, serological tests have confirmed *Brucella* infections in Ethiopian cattle, and a relatively high seroprevalence of brucellosis (above 10%) has been observed in smallholder dairy farms in central Ethiopia. Serological studies of brucellosis have been conducted in various locations across the country (Kassahun et al., 2010, Adugna et al., 2013). A higher seroprevalence of *Brucella* infection was found in intensive than in extensive production systems. The Borena zone reported the highest (50%) seroprevalence of *Brucella* (Dereje, 2022). Several reports have indicated that *Brucella* infection is common in Ethiopia (Minda et al., 2016). Bovine brucellosis serological studies from various researchers in the last two decades have shown that the disease is endemic and widespread (Table 1).

Table 1: Prevalence of Brucella Infection in Ethiopia.

Study Area	No. of Cattle	Breed	Test Type	Prevalences	References
Arsi Zone	370	Local	RBPT	0.1	(Degefa et al.,2011)
			CFT	0.1	
Ambo	169	Cross	RBPT	0.2	(Liya et al., 2015)
			CFT	0	
Assela	304	Mixed	RBPT	14.1	(Deselegn and Gangwar, 2011)
Central	1238	Mixed	RBPT	4.9	(Jergerfa et al., 2009)
			CFT	2.9	
Borana	283	Local	CFT	10.6	(Megersa et al., 2011)
East Showa	1106	Local	RBPT	11.2	(Hunduma and Regassa, 2009)
Eastern	435	Local	RBPT	1.8	(Degefa et al., 2011)
			CFT	1.4	
West	1152	Local	RBPT	1.2	(Adugna et al., 2013)
			CFT	1	
South East	862	Local	RBPT	1.4	(Gumi et al., 2013)
			CFT	1.4	
Debrezeit	300	Mixed	RBPT	3.0	(Fekadu et al., 2014)
			CFT	2.0	
Jimma Zone	1595	Mixed	RBPT	3.3	(Nuraddis et al., 2010)
			CFT	3.1	
Jimma zone	423	Mixed	CFT	4.3	(Dereje and Benti, 2020)
East Wollega	406	Mixed	RBPT	3.0	(Moti et al., 2012)
			CFT	2.0	
North Shewa	384	Cross	RBPT	0.8	(Pal et al., 2016)
North	1968	Local	CFT	4.9	(Mekonnen et al., 2011)
Central	1136	Mixed	RBPT	12.5	(Taye et al., 2008)
			CFT	11	
Afar	420	Both	RBPT	7.94	(Wossene and Teshager, 2021)
			CFT	3.5	
Somalia	116	Both	ELISA	6.9	(Tschopp et al., 2021)

Materials and Methods

Description of The Study Area

The study was conducted in four selected districts of the West Hararghe Zone in the Oromia Regional State of Ethiopia: Chiro, Daro Labu, Mieso, and Burka Dhintu. West Hararghe is located in the eastern part of Ethiopia, approximately 317 km from Addis Ababa. Geographically, the study area lies between 7°52'15" and 9°28'43" N latitude, and 40°03'33" and 40°34'13" E longitude, with an altitude ranging from 1,200 to 3,600 meters above sea level (Figure 1). The zone is characterized by three agro-climatic zones: highland (Dega), midland (Weina Dega), and lowland (Kola). Among these, Kola comprises the largest portion, covering 49.51% of the area, followed by Weina Dega at 38% and Dega at 12.49%. The area experiences two rainy seasons: kiremt/summer (June to September) and Belg/winter (February to April). Annual rainfall ranges from 650 to 1,500 mm, and the average temperature typically

falls between 20.5°C and 24°C. West Hararghe Zone consists of 17 districts (woredas), four of which Chiro, Daro Labu, Mieso, and Burka Dhintu were selected for this study. Of the total districts, four are considered pastoralist areas. A land use survey in the zone revealed the following distribution: 372,615 hectares are used for cultivation, 132,615 hectares are forest-covered, 3,362 hectares are marshy land, 64,665 hectares are bushland, 414,217 hectares are grazing land, 65,700 hectares are allocated for social services, and 366,981 hectares are degraded land. Livestock play a critical role in the crop-livestock mixed farming systems common in the area. Smallholder farmers rear various livestock species, including cattle, sheep, goats, chickens, camels, and equines. According to the West Hararghe Zonal Agriculture and Natural Resource Office (2016), the livestock population includes 1,017,806 cattle, 182,149 sheep, 890,226 goats, 216,819 donkeys, 1,102 mules, 1,512,784 chickens, 40,337 camels, and 65,846 beehives (Table 2).

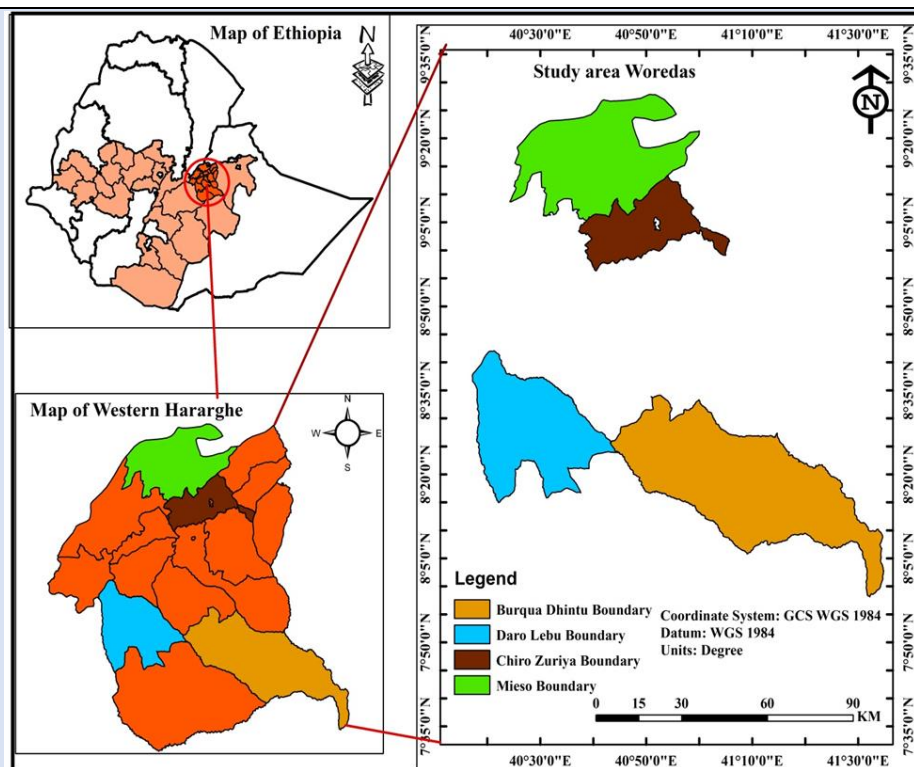


Figure 1: Map of the study areas (ESRI Arc GIS desktop 10.4 version software).

Study Population and Sampling Methods

The target populations for animals were Cattle in West Hararghe zone which are kept under different production system pastoral and agro pastoral systems. The study units were local cattle in four selected woreda in West Hararghe zone, which are kept under semi-intensive and extensive production system. Both sexes and age groups of more than six months of age were selected for the study (Table 2). Age was

estimated by examining their lower incisor teeth according to (Pace and Wakeman, 2003) and also by asking the owners, then and categorized as young (< 3 years), adults (4-7 years) and old (>8 years). Four woreda were purposively selected by easily accessibility, and the feasibility of sample collection; different agro-ecology, and altitude range management System and production system. The selected woreda Chiro, Mieso, Daro labu and Burka dhintu have 78, 108, 80 and 118 respectively.

Table 2: Cattle Population in the Study Areas.

No	Study Area (Woreda)	Cattle Population
1	Chiro	91,274
2	Mieso	125,915
3	Daro Labu	93,452
4	Burka Dhintu	136,860
5	Total	447,501

The numbers of animals to be sampled from each woreda were determined by the proportion of the cattle population existing in each woreda and kebeles. From each woreda, three kebeles selected the Simple Random Sampling method. From each kebele, three villages were selected. Starting from a presumed center of a village, a transect walk was done across the village together with an animal health worker. During transect traveling to different parts of the village, encountered households were contacted and their

animals were sampled randomly. A lottery system was used to select an individual animal from a herd, by assigning a code to the animal. Then those animals that had the chance for a number were selected for sampled until the required sample size for each kebele is fulfilled, so that nearly 50% of animals were selected from a herd. For the Knowledge, Attitude and Practice (KAP) study, occupationally associated 100 animals' owner, willing to be interviewed was included in Table 3.

Table 3: Proportion of Samples Collected from Each Selected Woreda and Kebele.

Woreda (District)	Kebeles	No. of Sample Colled	Sub Total
Chiro	Arba Rakete	24	78
	Alewagora	27	
	Wedeti	27	
Mieso	Gulufa	40	108
	Cacole	37	
	Fayoo	31	
Burka Dhintu	Rada Dibechea	42	118
	Burka Oda	40	
	Haro Ero	36	
Daro-Labu	Michata	25	80
	Rukessa	28	
	Sororo	27	
Total	12 kebeles	384	384

NO = Number of Animals

Study Design

A cross-sectional study was conducted from October 2023 to April 2024 G.C. to estimate the seroprevalence, assessment of KAP, and public health importance of bovine brucellosis in selected woreda of the West Hararghe zone Oromia regional state of Eastern Ethiopia

Sample Size Determination

The total number of animals required was determined using the formula given by (Thrusfield, 2018). The sample size was determined by using the 95% confidence interval at 50% expected prevalence and 0.05 desired absolute precision.

$$\text{Therefore, } n = \frac{(1.96)^2(p_{exp})(1-p_{exp})}{d^2} = 384$$

Where n = required sample size; p_{exp} = expected prevalence; d = desired absolute precision

Accordingly, 384 heads of cattle of both sexes and different age groups above 6 months of age were sampled from the Chiro, Daro labu, Mieso, and Burka dhintu woreda of the Western Hararghe zone. Subsequently, nearly all animal owners (N=100 respondents) whose cattle were sampled were included in the questionnaire survey.

Sample Collection and Transportation

During blood samples collection factors like age, sex, altitude, management System, herd size, and production system were collected. A sample collected early in the morning before animals are taken out for grazing. About 8-10 ml of blood samples was collected from each sampled animal via jugular-vein puncture using plain vacuotainer tubes and needles after proper restraining and disinfecting of the site using 70% alcohol. Each sample were labeled with specific standard identification and transported to Hirna Regional Veterinary Laboratory using an icebox for

further processing. the tubes were set tilted overnight at room temperature to allow clotting and the next morning, sera were harvested from the clotted blood by sterile and labeled cryovial tubes. which were labeled and stored in a deep freezer until tested. all serum samples were screened using Rose Bengal Plate Test (RBPT) at Hirna Regional Veterinary Laboratory and positive samples were tested at Hirna Regional Veterinary Laboratory using I-ELISA Test.

Serological Tests Screening and Confirmation

Serology Test: The Modified Rose Bengal Plate Test (mRBPT) was employed as a screening test on the serum samples for the presence of Brucella agglutinins. Positive sera were then retested for confirmation by the indirect enzyme-linked immunosorbent assay (I-ELISA) Hirna regional laboratory.

Modified Rose Bengal Plate Test (mRBPT)

All collected sera samples were screened using the Rose Bengal Plate Test in Hirna Regional Veterinary Laboratory as per the procedure described by (OIE, 2004). Accordingly, of the 384 sera tested by RBPT, 15 sera were found positive by RBPT. the following procedures were for the Rose Bengal Plate Test: a drop of serum sample was placed on a clean grease-free ceramic tile and a 30 µl volume of antigen was placed near the 30 µl serum sample. The serum and antigen were mixed thoroughly using a sterile wire loop to produce a circular or oval zone approximately 2cm in diameter. The mixture was agitated gently by shaking for 4 minutes at ambient temperature (25°C) by gently rocking the slide. The results were read by Samples showing the presence of agglutination (even slight) are considered positive samples (presence of anti-brucellosis antibodies) were recorded as positive and Samples showing no agglutination are considered

negative samples (absence of anti-brucellosis antibodies). *No Agglutination*: Absence of antibodies (negative). *Agglutination* (even slight) presence of antibodies (positive). Its positive results should be confirmed by a more specific test such as the I-ELISA test. (ID Vet, 310, innovative Diagnosis, France).

Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA)

All RBPT-positive tested sera were retested using ELISA for further confirmation at Hirna regional veterinary laboratory, Hirna, Oromia, Ethiopia. Those 15 RBPT-positive sera were further subjected to the more specific ELISA test and as a result, 12 sera were confirmed to be *Brucella* positive by the ELISA test. In direct ELISA detection antibodies of brucellosis were performed at Hirna Regional Veterinary laboratory. The technique was performed according to the instructions from the manufacturer (ID Vet, 310, innovative Diagnosis, France). In brief, add 190 µl of dilution buffer to all wells, add 10 µl of negative control to wells A1 and B1, add 10 µl of positive control to wells C1 and D1, and add 10 µl of sample serum to the left wells. incubate for 45 min at 21-degree centigrade. wash each well 3 times with 300 µl of the wash solution. add 100 µl of the conjugate to each well. incubate for 30 min at 21-degree centigrade. wash each well 3 times with 300 µl of the wash solution. add 100 µl of the substrate solution to each well. incubate for 15 min in the dark. add 100 µl of the stop solution to each well in order to stop the reaction. read and record the OD at 450 nm. Then for each sample calculate the percentage of inhibition (S/P %). If the percentage of inhibition was less than or equal to 120 the result was negative and if the percentage of inhibition was greater than 120 the result was positive

Questionnaires

A structured interview question was asked to 100 animal workers in the study area who had direct contact with the animal or its products. The survey collected information on the knowledge, attitude, and practice of the respondents about brucellosis and its public health impact. The questionnaire included questions on the consumption of raw milk, the handling of aborted materials, and the history of abortion, chronic headache, knee pain, and testicular swelling among the participants. The data were analyzed to identify the risk factors and preventive measures for brucellosis. The questionnaire also

gathered data on personal demographics such as age, gender, education level, knowledge, attitude, and practice about brucellosis, raw milk consumption, handling of aborted materials, history of abortion, chronic headache, knee pain, and testicular swelling to assess the public health impact. The occurrence of abortion, stillbirths, retention of fetal membranes, and contact between animals from different herds were recorded. The breeding methods were classified by service types (artificial insemination (AI), bull, or both). The disposal methods of after-birth materials (placenta, aborted material, and dead fetus) were also noted as burying, eaten by a dog, or thrown into an open dump. The questionnaire survey for Commonly Knowledge, attitude, and Practice on bovine brucellosis used the formula given by (Arsham, 2002) to calculate the sample size. which is:

$$N = \frac{0.25}{SE^2} = \frac{0.25}{0.0025} = 100$$

Where N= Sample Size; SE (Standard Error) = 5%

Data Management and Analysis

The responses from the questionnaire survey and the results from the laboratory tests were entered and organized in a Microsoft Excel spreadsheet (Microsoft Corporation). We used descriptive statistics to describe the demographic features and the knowledge, attitude and practice (KAP) of brucellosis among the participants. We used SPSS26 version for Windows to perform a crosstab analysis for the seroprevalence of bovine brucellosis. We applied the Chi-square (χ^2) test to compare the seroprevalence of bovine brucellosis with various factors related to bovine brucellosis, such as age, altitude, flock size, woreda, kebele and production system, management system. We calculated the seroprevalence as the ratio of seropositive samples to the total number of samples tested.

Results

seroprevalence of bovine brucellosis was computed by descriptive statistics. Of 384 samples tested by RBPT, 15 were found to be positive with a seroprevalence of brucellosis at 3.9%. Since RBPT is a less specific test for *Brucella* antibody detection, those 15 RBPT-positive samples were further subjected to a more specific and confirmatory test, the I-ELISA test. As a result, of 15 RBPT-positive samples retested by I-ELISA, 12 were found positive for *Brucella* providing an overall seroprevalence of 3.1%. The results are summarized in Table 4 of the study.

Table 4: The overall seroprevalence of bovine brucellosis in the study area.

Tests Type	Total No. of Animals Tested	Total No. (%) of Positive Animals
RBPT	384	15 (3.9%)
ELISA	15	12(80%)
Total	384	3.1%

No =Number of Animals; RBPT= Rose Bengal Pate Test; ELISA= Enzyme-Linked Immuno Sorbent Assay; S = Serological

Seroprevalence of Bovine Brucellosis in Woreda Level

15 animals (3.9%) responded positively to brucellosis out of 384 sera tested using RBT. These reactors were further tested again using I-ELISA and 12 animals (3.1%) were confirmed to be seropositive for brucellosis. The highest prevalence rate was found in

the Burka Dhintu woreda (5.1%). The second prevalence rate was found in the Mieso woreda (3.7%), with the lowest seroprevalence rates in Daro Labu (1.3%) and Chiro (1.3%). Daro Labu and Chiro woreda had similar seroprevalence rates, but the difference in prevalence between woreda was not statistically significant ($P>0.05$). The results are summarized in Table 5 of the study.

Table 5: The overall seroprevalence of bovine brucellosis in study woreda.

Woreda	No of tested	mRBPT, no of positive (%)	I-ELISA, no of positive (%)
Burka Dhintu	118	7(5.9%)	6(5.1%)
Mieso	108	5(4.6%)	4(3.7%)
Daro Labu	80	2(2.5%)	1(1.3%)
Chiro	78	1(1.3%)	1(1.3%)
Total	384	15(3.9%)	12(3.1%)

PSP =Percent Seroprevalence; RBPT= Rose Bengal Pate Test; I-ELISA= Inderect Enzyme-Linked Immuno Sorbent Assay; No = Number

Seroprevalence of Bovine Brucellosis and Different Variables

Based on the study, the difference in bovine brucellosis seroprevalence among different age groups was statistically significant ($P>0.05$). The seroprevalence of the disease was found to be 0.5%, 0.8% and 1.8% in adolescents, adults, and elderly, respectively. Older age was more exposed compared to younger and adult groups. It is also significant to be more seropositive compared to other variables.

The sex of the studied animals had no significant ($P>0.05$) correlation with the seroprevalence of bovine brucellosis. In this study, the seroprevalence of bovine brucellosis was recorded (2.6%) in large herd sizes and 0.5% in small herd sizes. This indicates that

animals kept in large herd sizes were more likely to be exposed to Brucella infections than small herds. It is also statistically insignificant ($P>0.05$). The seroprevalence of the disease in female and male cattle of 2.9% and 0.3%, respectively, was recorded in the study. Pastoral Production Systems are riskier than Agro pastorals. Extensive management systems are more risky than semi-intensive management systems. kebeles in the study area, the highest seroprevalence was recorded in Burka oda (1.3%) and Gulufa (0.8%), while the lowest was recorded in Rukesa, Fayoo, Wedeti, Rada dibicha (0.3%).and the Sororo, Michata, Arbarakate, Alowgora, Cacole and Haro Ero had zero prevalence (0%) recorded. However, the prevalence among kebele was statistically insignificant ($P>0.05$).

Table 6: Distribution of General Farm Characteristics and their Association with the Prevalence of Bovine Brucellosis.

Variable	Category	No. Tested	MRBPT Positive n (%)	ELISA Positive n (%)	P-Value
Sex	Male	90	2 (2.2%)	1 (1.1%)	0.210
	Female	294	13 (4.4%)	11 (3.7%)	
Age	Young	97	3 (3.1%)	2 (2.1%)	0.010
	Adult	201	4 (2.0%)	3 (1.5%)	
	Old	86	8 (9.3%)	7 (8.1%)	
Woreda	Burka Dhintu	118	7 (5.9%)	6 (5.1%)	0.331
	Mieso	108	5 (4.6%)	4 (3.7%)	
	Chiro	78	1 (1.3%)	1 (1.3%)	
	Daro Labu	80	2 (2.5%)	1 (1.3%)	
Kebele	Sororo	27	0 (0.0%)	0 (0.0%)	0.046
	Michata	25	0 (0.0%)	0 (0.0%)	

	Rukessa	28	2 (7.1%)	1 (3.6%)	
	Arba Rakete	27	0 (0.0%)	0 (0.0%)	
	Wedeti	27	1 (3.7%)	1 (3.7%)	
	Alewagora	24	0 (0.0%)	0 (0.0%)	
	Cacole	37	0 (0.0%)	0 (0.0%)	
	Gulufa	40	3 (7.5%)	3 (7.5%)	
	Fayoo	31	2 (6.5%)	1 (3.2%)	
	Rada Dibechea	42	1 (2.4%)	1 (2.4%)	
	Burka Oda	40	6 (15.0%)	5 (12.5%)	
	Haro Ero	36	0 (0.0%)	0 (0.0%)	
Prodn System	Agro-pastoral	158	3 (1.9%)	2 (1.3%)	0.080
	Pastoral	226	12 (5.3%)	10 (4.4%)	
Altitude	Lowlands	254	14 (5.5%)	11 (4.3%)	0.152
	Midlands	78	1 (1.3%)	1 (1.3%)	
	Highlands	52	0 (0.0%)	0 (0.0%)	
Herd Size	Small (<10)	177	4 (2.3%)	2 (1.1%)	0.038
	Large (>10)	207	11 (5.3%)	10 (4.8%)	
Man. System	Semi-intensive	130	1 (0.8%)	1 (0.8%)	0.023
	Extensive	254	14 (5.5%)	11 (4.3%)	

NO= Number of Animals; RBPT= Rose Bengal Pate Test; I-ELISA= Enzyme-Linked Immuno Sorbent Assay

Socio-Demography of the Respondents

The study involved 100 people from four different woredas in Ethiopia. Chiro and Daro Labu each had 26 participants, while Mieso and Burka Dhintu had 23 and 25 participants, respectively. Most of the participants (60%) could not read or write, and only 10% had some formal education. The age range was 29% under 30 years, 61% between 31-50 years, and

10% over 50 years. There were more men (72%) than women (28%) in the sample. The main livelihoods of the participants were either agricultural pastoralism (52%) or pastoralism (48%). Most of them (88%) were married and had dependents. One of the potential risks for the spread of the disease was the reliance of many farms on external sources for livestock replenishment (Table 7).

Table 7: Descriptive Statistics of Survey Respondents (n = 100).

Socio-demographic Characteristics	Category	Frequency (n)	Percentage (%)
Gender	Male	72	72%
	Female	28	28%
Woreda	Chiro	26	26%
	Mieso	23	23%
	Burka Dhintu	25	25%
	Daro Labu	26	26%
Education Level	Illiterate	60	60%
	Read and write	30	30%
	Formal schooling	8	8%
	Educated	2	2%
Kebele	Arba Rakete	9	9%
	Alewagora	8	8%
	Wedeti	9	9%
	Cacole	9	9%
	Gulufa	8	8%
	Fayoo	6	6%
	Rada Dibechea	8	8%
	Burka Oda	10	10%
	Haro Ero	7	7%
	Michata	8	8%
	Sororo	9	9%
	Rukessa	9	9%
Occupation of Household	Pastoral	48	48%
	Agro-pastoral	52	52%
Age of Respondent	< 30 years	29	29%
	31–50 years	61	61%

	> 50 years	10	10%
Marital Status	Married	88	88%
	Single	12	12%
Do You Have Any Cattle?	Yes	100	100%
	No	0	0%
Source of Cattle	Bought from people in the community	19	19%
	Inherited and/or gift	46	46%
	Bought from other areas	35	35%
Herd Size	Small (< 10)	59	59%
	Large (> 10)	41	41%

Knowledge on Brucellosis as Disease in Cattle and in Humans

This study revealed that the majority of respondents, 80 (80%), had no information about brucellosis, while 20 (20%) of respondents had information and knowledge about brucellosis. The main sources of information on brucellosis were veterinary services (17%), community gatherings or talks (1%), friends or family members (1%), and radio or television (1%).

When asked about the zoonotic nature of brucellosis, most of the respondents, 83 (83%), mentioned that they had poor knowledge of zoonotic diseases, while 17 (17%) of respondents were well informed on the zoonotic importance of bovine brucellosis. Only 17% of respondents knew that brucellosis can be transmitted from cattle to humans, while 83% were unaware.

When asked about the transmission of brucellosis in cattle, 80% (80/100) of respondents did not know, 10% (10/100) stated abortion, 3% (3/100) stated placenta from live births, and 7% (7/100) stated shared grazing. Participants reported the following as signs of brucellosis in cattle: abortion (19%), weak

calves (0%), bull infertility (1%), and hygroma/swelling of joint (7%). The rest of the respondents (80%) did not know any signs of brucellosis in cattle.

Among the respondents who knew about the zoonotic transmission of brucellosis, drinking raw milk (5%), assisting with calving or handling placenta (3%), slaughtering an infected animal (0%), handling an abortion (0%), and both drinking raw milk and assisting with calving or handling placenta (9%) were reported as possible means of transmission.

When asked about the symptoms of brucellosis in humans, 83% (83/100) of participants did not know, and 7% (7/100) cited fever, 1% (1/100) cited headache, 1% (1/100) cited flu-like symptoms, 2% (2/100) cited testicular swelling, 2% (2/100) cited sterility, and 4% (4/100) cited both fever and headache. Finally, 45% of respondents observed abortion history in their animal flocks, while 55% did not. Among those who observed abortion history, 11% reported that abortion occurred at late stage of pregnancy, 25% at mid stage, and 9% at early stage which are shown in (Table 8).

Table 8: Knowledge of Brucellosis Among Participants Aware of the Disease in the Community.

Knowledge of Brucellosis	Response	Frequency (%)
Awareness about Brucellosis	Yes	20 (20%)
	No	80 (80%)
Knowledge that Brucellosis is Zoonotic	Yes	17 (17%)
	No	83 (83%)
Where Did the Participant Hear of Brucellosis	Veterinary services	17 (17%)
	Community gatherings/talks	1 (1%)
	Neighbours/friends/family	1 (1%)
	Radio/television	1 (1%)
	No knowledge of Brucellosis	80 (80%)
History of Abortion	Yes	45 (45%)
	No	55 (55%)
Stage of Abortion	1-3 months	11 (11%)
	4-6 months	25 (25%)
	More than 7 months	9 (9%)
	No history of abortion	55 (55%)
Transmission of Brucellosis in Cattle	Don't know	80 (80%)
	Abortion	10 (10%)
	Placenta from live births	3 (3%)

	Shared grazing	7 (7%)
Symptoms Appear in Cattle	Don't know	80 (80%)
	Abortion	19 (19%)
	Bull infertility	1 (1%)
Transmission of Brucellosis in Humans	Drinking raw milk	5 (5%)
	Assisting calving/placenta	3 (3%)
	Both	9 (9%)
	Don't know	83 (83%)
Symptoms in Humans	Don't know	83 (83%)
	Fever	7 (7%)
	Headache	1 (1%)
	Flu-like symptoms	1 (1%)
	Testicular swelling	2 (2%)
	Sterility	2 (2%)
	Both	4 (4%)
	Don't know	83 (83%)
Animal With Joint Swelling	Yes	7 (7%)
	No	93 (93%)

Respondents' Practices Relating to Meat and Dairy Consumption and Cattle Husbandry

In this study, the majority of respondents reported that they consumed raw milk (73%) and raw or soured milk (40% and 33%, respectively). Only 27% of respondents consumed boiled and/or pasteurized milk. Most of the respondents also indicated that they performed several risky practices frequently, such as consuming raw milk (73%) and assisting during parturition (92%). However, they did few preventive practices, such as cooking meat and boiling milk before consumption (27%).

Regarding assistance during parturition, 47% of respondents stated that a male household member had helped to deliver a calf, 34% stated that a female household member had helped, 1% stated that a veterinarian had helped, and 18% stated that both male and female household members had helped. Furthermore, 45% of respondents stated that a household member had contact with aborted fetus and placental membranes. When asked what action

would be taken if an aborted fetus was found, 30% of respondents stated that they would give it to a dog, 50% stated that they would throw it to an open dump, 16% stated that they would do nothing, and 4% stated that they would burn or bury it. Only 37% of respondents stated that they cleaned the parturition area after birth, while 63% did not.

Most of the respondents (87%) in this study used bulls for service. All these respondents were from the extensive management system and had no other options, while 13% of the farmers in the semi-intensive management system had the opportunity to use artificial insemination. In addition, most of the respondents (55%) did not wash their hands with water and soap after contact with animals or animal products, and only 3% disinfected their hands with alcohol after washing. These factors, combined with the unawareness of most of the farmers on brucellosis and the poor cleaning and disinfection practices, could increase the risk of transmission of brucellosis between animals and humans. Which are shown in (Table 9).

Table 9: Practices of Participants Regarding Dairy Consumption and Cattle Husbandry in the Community.

Practices of Brucellosis	Response	Frequency (%)
Do you help the animal during birth	Yes	92 (92%)
	No	8 (8%)
Was there any occurrence of abortion in your farm	Yes	45 (45%)
	No	55 (55%)
How many miscarried fetus and placenta encountered	Once	15 (15%)
	Twice	16 (16%)
	Many Times,	14 (14%)
	No	55 (55%)
Contact with aborted fetus	Yes	45 (45%)
	No	55 (55%)
Hand washing after contact with aborted fetus	Yes	45 (45%)
	No	55 (55%)
Do you disinfect hands with alcohol after washing	Yes	3 (3%)

	No	97 (97%)
Does your family drink uncooked cow's milk	Yes	73 (73%)
	No	27 (27%)
Condition of meat and milk consumed	Boiled	27 (27%)
	Raw	40 (40%)
	Raw and soured	33 (33%)
Who assists during kidding/lambing?	Male	47 (47%)
	Female	34 (34%)
	Veterinarian	1 (1%)
	Both(F/M)	18 (18%)
Action taken if placental membrane is found	Burying and burning	4 (4%)
	Give to dogs	30 (30%)
	Throw or dump	50 (50%)
	Nothing	16 (16%)
Breeding methods	AI	0 (0%)
	Bull	87 (87%)
	Both AI and Bull	13 (13%)
Cleaning parturition area after birth	Yes	37 (37%)
	No	63 (63%)

Attitudes Towards Animal Health, Human Health and Brucellosis

Of the respondents who had heard of brucellosis, 20 (20%) believed it was an important public health disease, and only 1% believed that some of their family members were at risk of contracting brucellosis. Every one of those considered the person in the household working most with the cows exposed to the highest risk. The majority of participants, 80 (80%) and 83 (83%), did not believe that brucellosis was an important public health disease and did not consider their family at risk of acquiring a brucellosis disease, respectively.

When asked if they ensured that new cattle were healthy before buying or receiving them, 100% (100/100) responded positively. To ensure cattle health, 2% (2/100) of respondents stated that they sought veterinary advice, 21% (21/100) bought from people they knew or trusted, and 77% (77/100) relied on their own experience. When asked what action

would be taken if a cow aborted, 61% (61/100) of respondents indicated that they would contact veterinary services for help, 17% (17/100) would treat with home remedies, 2% (2/100) did not know what to do, and 20% (20/100) would do nothing.

Regarding brucellosis being treatable in animals and humans, 17 (17%) believed it was treatable in both animals and humans, while 83 (83%) believed it was not treatable in both animals and humans. For the questions of prevention of brucellosis in animals and humans, 17 (17%) responded that brucellosis can be prevented in animals and humans, while 83% responded that brucellosis cannot be prevented in animals and humans. However, the majority of the respondents (83%) did not know that boiled milk and cooking meat could prevent brucellosis in animals and humans. Most of the herders (83%) reflected that they had no information on brucellosis, while only 17% had full information on brucellosis which are shown in Table 10.

Table 10: Participant Attitudes toward Animal Health, Human Health, and Brucellosis in the Study Area Community.

Attitudes	Response	Frequency (%)
Brucellosis is an important public health disease in Ethiopia	Yes	17 (17%)
	No	83 (83%)
How is health ensured when receiving new cattle?	Seek veterinary advice	2 (2%)
	Buy from people known and/or trusted	21 (21%)
	Rely on own experience	77 (77%)
Are any family members at risk of acquiring brucellosis?	Yes	1 (1%)
	No	99 (99%)
Action taken if a cow abort	Contact veterinary services	61 (61%)
	Don't know	2 (2%)
	Nothing	20 (20%)
	Treat with home remedies	17 (17%)
	Yes	20 (20%)

Do you think cooking meat and boiling milk prevents brucellosis?	No	80 (80%)
Do you think brucellosis is treatable in animals and humans?	Yes	17 (17%)
	No	83 (83%)
Do you think brucellosis can be prevented in animals and humans?	Yes	17 (17%)
	No	83 (83%)

Discussion

The overall sero prevalence of brucellosis in individual animals was 3.1 % (n=384) in the study area in both extensive and semi-intensive management system. this indicates that brucellosis is an endemic disease in the region and poses a significant threat to animal and human health. Overall seroprevalence of 3.1% was comparable to the findings of several other authors in Ethiopia such as the seroprevalence in this study was higher than the finding of 1.66% in Sidama zone (Kassahun et al., 2010), 1.38% in Jijiga zone (Degefu et al., 2011), 1.97% in East Wollega zone (Moti et al., 2012), 1% in west (Adugna et al., 2013), 1.94% in bench Manji zone (Tigist and Bekele, 2021), 2.0% from Debrezeit (Fekadu et al., 2014).

The overall seroprevalence of 3.1% is comparable to some previous studies conducted in Ethiopia but lower than others. This variation could be attributed to differences in sample size, diagnostic methods, geographical location, and management practices. it is lower than other reports from Ethiopia such as 3.5% from pastoral and mixed farming (Bekele et al., 2011), 5.7% in Afar (Wossene and Teshager, 2021), 11.2% from East Showa (Hunduma and Regassa 2009), 11% from central (Taye et al., 2008), 11.6% from Jimma zone (Dereje and Benti, 2020), 10.6 % in Borana (Bekele et al., 2011), 8.0% from Borana area (Bekele et al., 2012), 6.1% from Tigray, 4.9% from north (Mekonnen et al., 2011). This study reported an overall prevalence of 3.1 % in cattle in extensive and semi-intensive management in selected areas of west Harerghe Zone. The prevalence in this study was in agreement with the findings of (Nuraddis et al., 2010) prevalence of 3.1 % in cattle in extensive and intensive management in the Jimma zone.

In this study, there was a significant association between the age categories of the tested animals and seroprevalence of bovine brucellosis. Infected animals were young, adult and old age. Significantly higher seroprevalence was observed in the older age category than the younger age and adult age category. The seroprevalence rates were 58.3% (1.8%) for old age, 25% (0.8%) for adult age and 16.7% (0.5%) for young age respectively. This observation is agreement study

in Gambia by (Unger et al., 2003) in which 52 % of the seropositive cows were older than 6 years. (Moti et al., 2012), older age group were affected more than younger animals ($P < 0.05$) in the extensive management system, (Bekele et al., 2000) and (Taye et al., 2008) also reported similar results that indicate a higher prevalence in animals above six years of age. it has been reported that susceptibility of cattle to *Brucella* infection is influenced by age of the individual animal. thus, sexually matured and pregnant cattle are more susceptible to infection with *Brucella* organisms than sexually immature animals of either sex, this may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Tegegn et al., 2016).

In this study, only 0.3% of males had a lower prevalence than females (2.9%) for ELISA tests. Sex has been one of the risk factors affecting susceptibility of cattle to *B. Abortus* infection. It is well known that female cattle are more susceptible to *Brucella* infection than males. Similar to the result of the present study, a higher seroprevalence of bovine brucellosis in females than males were recorded by (Tadele et al., 2010). The reason was explained by (Taye et al., 2008) that males are kept for relatively shorter time duration in breeding herd than females and thus the chance of exposure is lower for males. agro-ecological, there is not statistically significant but lowland altitude is higher prevalence on the study area. According to a study, the seroprevalence of *Brucella abortus* in cattle was 2.9% in the lowlands, 0.3% in the midlands and 0.0% in the highlands this indicates that cattle in the lowlands are more exposed to *Brucella abortus* than those in the higher altitudes (Edao et al., 2020).

In this study, 2.6% large herd sizes and 0.5% small herd sizes, seroprevalence were higher in larger herd sizes. this are statistically significant difference was also observed in the seroprevalence of bovine brucellosis among different herd sizes in study areas. animals from large herds were at greater risk of brucellosis in the study areas. this observation is in agreement with the previous findings of other authors

(Tadele, 2004) reported a higher prevalence of brucellosis in larger herds than smaller herds. It was also indicated by (Nowak and Walker, 1999) that herd size and animal density are directly related to prevalence of disease and difficulty in controlling infection in a population. In addition (Kassahun et al., 2010, Hellmann et al., 1984) indicated that herds of bigger size had higher seroprevalence of bovine brucellosis as compared to smaller herds. In larger herd sizes, the disease spreads by several modes of transfer, especially through contact with infected discharges from dam and its fetus (Tegegn et al., 2016). In Ethiopia, different parts of the country have different reports on how common brucellosis is. This study found higher prevalence in Burka dhintu (5.1%) and Mieso (3.7%) districts. These districts have extensive management systems, where animals are kept in large groups and graze together. This may increase the contact and transmission of the disease among animals and humans.

The finding of this study revealed that relatively higher seroprevalence of brucellosis was observed in extensive than semi-intensive management systems. 2.9% extensive management systems and 0.3% semi-intensive management systems. This finding observed in the extensive management system could be partly explained by the fact that contact between animals increases in communal grazing practices which was the predominant feeding system in the extensive type of management. In such circumstances, cattle of unknown disease status might mix and often graze together and resulting in the spreading and transmission of disease among herds. About 52% of the study area shared the communal grazing system. Similar findings were indicated in Sri Lanka by (Silva et al., 2000). where free grazing which allows unrestricted contact between animals may contribute to the spread of brucellosis in extensive management systems (Megersa et al., 2011).

The lowest and similar seroprevalence was recorded in semi-intensive areas of the Chiro and Daro-labu district while only 0.6% was seropositive in two districts in the study areas. This is due to animals being kept separately and tethered around the homestead and feeding on post-harvest products. These areas are partly cash crops (khat, different crop production like sorghum) growing region of the country where small numbers of animals are kept separately. similar findings of low seroprevalences were reported from crop-livestock mixed farming areas of Eritrea (Omer et al., 2000) and Ethiopia

(Gebretsadik et al., 2007). likewise, the use of maternity pens at calving is proved to be associated with a decrease in prevalence of infection, presumably due to decreasing the exposure of infected and susceptible animals (Liya et al., 2015).

According to a questionnaire survey conducted in Chiro, Daro lebu, Mieso, and Burka dhintu most of the respondents practice contacting abortion and placental retention. After contact, only 45% of the respondents wash their hands, and only 3% disinfect their hands with alcohol after washing. 97% of the respondents do not use alcohol to disinfect their hands, and 100% of the respondents in the study area do not use personal protective equipment. These practices play a major role in the spread of *Brucella*. In addition, most of the respondents in this study did not bury them afterbirth (aborted fetus, stillbirth, and retained fetal membrane) rather they left them. 50% of the respondents left them in an open field, 30% gave them to dogs, and 16% did nothing. This study in general shows poor knowledge, poor practice, and poor attitudes toward brucellosis and public health. Comparable studies have also reported similar findings. The owners' lack of proper hygiene after handling aborted materials (91.2%) could increase the likelihood of transmitting the infection to healthy animals (Tadele, 2004). The majority of the people who raised animals were unaware of the basics of brucellosis, such as how it can infect humans through eating uncooked meat or milk, touching aborted animals or their fluids, and how to prevent it. About 80% of the respondents in the study area lacked any knowledge of brucellosis.

This is similar to findings of previous studies done in Kenya, (Namanda et al., 2009) 80% of respondents were no knew of the existence of brucellosis whereas in Tajikistan 85% of the respondents had never heard of brucellosis (Lindahl et al., 2015) 83% of the respondents from heard owner relatively did not understand the methods of zoonotic disease transmission including brucellosis. community lack of awareness about brucellosis, improper handling of aborted materials, and the habit of consuming raw milk, among other factors, might contribute to the further spread of brucellosis in their livestock and expose the community to a public health hazard (Megersa et al., 2011).

Generally, results of herders' knowledge, attitude, and practices about brucellosis showed that most of the livestock keepers have poor knowledge about brucellosis and its transmission to humans through

consumption of raw milk, raw meat, contact with aborting fetus and discharge as well as its prevention measures. 80% of the respondents had no information about brucellosis, 73% had risk practices, and 83% had negative attitudes respectively other studies similar to this. Out of 300 respondents, 240 (80%) had no information about brucellosis, 249 (83%) had risk practices, and 249 (83%) had negative attitudes respectively in among Mongolian (baljinnyam, 2014, bat-erdene et al., 2019).

Conclusion and Recommendations

In the study conducted in West Harerge Zone four selected woreda, the seroprevalence of bovine brucellosis in individual animals was 3.1% (n=384) in both extensive and semi-intensive management systems. The observed seroprevalence of animals at 3.1% in the study area can be regarded as medium prevalence and has risk public health significance. Variables such as herd size, age, kebele and management systems seem to be important variables that should be considered in disease control. Results of assessment of KAP and public health implication interviewer 100 animal owners showed that most of the livestock keepers had poor knowledge about brucellosis and transmission of brucellosis to humans through consumption of raw milk, raw meat, contact aborting fetus and discharge as well as its prevention measures. By percent, where 80% of the respondents had no information about brucellosis, 73% had risk practices, and the something 83% has to negative attitudes respectively. Livestock keepers of the study area had poor basic knowledge about bovine brucellosis, its importance on public health, and insufficient knowledge of disease prevention measures. enhanced public health education on the cause, symptoms, and mode of transmission of brucellosis would be important for the prevention and control of the disease in the present study area. based on the above conclusions, the following recommendations are forwarded to curb the further spread of the disease in both cattle and human populations:

- Educate the community about brucellosis and how to avoid it. Use informal channels like radio, posters and etc.
- Through the government, veterinary workers and health extensions should sit down together and schedule at least once a month to provide awareness to the community.

- Strict movement control of animals from one area to another in order to prevent the spread and transmission of the disease from infected cattle to non-infected ones.
- Proper hygienic practices and good husbandry management should be exercised and these could in many situations minimize the spread of disease in the herd.

Declarations

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Approval and Consent to Participate

Ethical approval for this study was obtained from the College of Veterinary Medicine, Haramaya University. Prior to sample and data collection, the objectives and procedures of the study were clearly explained, and official permission was obtained from the relevant district agricultural offices as well as animal owners. Verbal informed consent was obtained from all participants after assuring them that their personal information would be kept strictly confidential and that participation was entirely voluntary, with the right to withdraw at any point.

Consent for Publication

We have read and approved the final manuscript, and consent is given for its publication in this journal.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

We declare that we have no competing interests.

Funding Declaration

We did not receive any funding, grants, or financial support from any public, private, or non-profit organizations for the conduct of this research.

Authors' Contributions

DT conceptualized the research idea. MA and DT collaboratively planned and designed the structure of the study. MA and DT wrote the initial manuscript, while DT provided revisions, addressed comments, and edited the manuscript. All authors read and approved the final version of the manuscript.

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