BRUCELLOSIS: SEROPREVALENCE, KNOWLEDGE, ATTITUDE AND EXPOSURE RISKS OF HERDERS' IN SOMALI REGION, ETHIOPIA

BY:

ABDULLAHI ADAN AHAD

DEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND PUBLIC HEALTH (MIVPH)

MASTER OF VETERINARY SCIENCE IN VETERINARY PUBLIC HEALTH PROGRAM

JUNE, 2022

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ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

As MVSc research advisor I hereby certify that I have read and evaluated this thesis prepared under my guidance by **Abdullahi Adan Ahad** entitled: "**BRUCELLOSIS: SEROPREVALENCE, KNOWLEDGE, ATTITUDE AND EXPOSURE RISK OF HERDERS' IN SOMALI REGION, ETHIOPIA".**

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As a member of the examining board of the final MVSc open defense, we certify that we have read and evaluated the thesis entitled **"BRUCELLOSIS: SEROPREVALENCE, KNOWLEDGE, ATTITUDE AND EXPOSURE RISKS OF HERDERS' IN SOMALI REGION, ETHIOPIA".** Prepared by **Abdullahi Adan Ahad** and recommend that it will be accepted as fulfilling the thesis requirement for the Degree of **Master of Science in Veterinary Public Health**.

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STATEMENT OF THE AUTHOR

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

Name:Abdullahi Adan Ahad Signature:_____

Date of Submission: 16/06/2022

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LIST OF ABBREVIATIONS

AAU-CVM	Addis Ababa University College of Veterinary Medicine and Agriculture
BCV	*Brucella* Containing Vacuole
cELISA	competitive Enzyme Linked Immune Sorbent Assay
CFSPH	Center of Food Security and Public Health
CFT	Complement Fixation Test
CSA	Central Static Agency
FPA	Fluorescence Polarization Assay
HHs	Households
IgG	Immunoglobulin G
KAP	Knowledge, Attitude, and Practice
LPS	Lipopolysaccharide
MZN	Modified Ziehl-Neelsen
OIE	World Organization for Animal Health
PA	Peasants Associations
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
SAT	Slow Agglutination Test
SRBoFED	Somali Region Bureau of Finance, Economic and Development
STATA	South Texas Art Therapy Association
T4SS	Type IV Secretion Systems

ABSTRACT

Brucellosis is a contagious bacterial disease caused by the genus *Brucella* that poses a major public and animal health problem in many parts of the world, particularly in pastoral settings where livestock and livestock products are the primary source of income. A cross-sectional study was conducted from December, 2021 to April, 2022, to estimate the prevalence and associated risk factors of livestock and human brucellosis, as well as to assess herders' knowledge, attitude, and exposure risk in three selected districts of Somali region, Ethiopia. A total of 1000 blood samples were collected from goats (n=201), sheep (n= 99), camel (n= 450) and human (n=250). The serum samples were screened with Rose Bengal plate test (RBPT) and positive ones were further confirmed using competitive Enzyme Linked Immino Sorbent Assay (cELISA). The results showed that 45 (6.0%) and 28 (3.7%) of the animal samples tested positive for RBPT and cELISA, respectively. Samples from occupationally linked human, on the other hand, were confirmed positive 9(3.6%) by RBPT and 5(2.0%, 95% CI, 0.6-4.6) by cELISA. Goats had the highest seroprevalence (6.5%; 95% Cl, 3.5-10.8), followed by camels (2.9%; 95% Cl, 1.5-4.9) and sheep (2.0 %, 95%CI, 0.2-7.1). Sex, herd size, and herd history of abortion, all showed a significant association with *Brucella* seropositivity logistic regression analysis. Furthermore, in multivariable Firths' logistic regression analysis for human samples, factors such as, gender, presence of fever or joint pain, and involvement in disposal of aborted or retained fetal membrane (RFM) were statistically significant ($P<0.05$). About half of the herders had heard about the disease (48%), of whom very low proportion (17%) knew brucellosis is a zoonotic disease. Despite respondents' poor knowledge and high involvement in risky practices, significant positive attitudes toward disease prevention were identified in this study. In conclusion, this study provides a baseline information for the implemention of feasible disease control strategy in terms of pastoral community and sociocultural status through one health approach.

Keywords: *Brucellosis; KAP; Seroprevalence; Somali region ; Zoonosis*

1. INTRODUCTION

Brucellosis is an infectious bacterial disease caused by the genus *Brucella*. Scientist, Bruce isolated from spleen of a soldier dying for the first time in 1887 at malta and named it *Micrococcus Melitensis* (Luelseged, 2018). There are eight terrestrial species and two marine species among the ten known *Brucella* species. The second most common cause of human infection is *B. abortus*, which is found primarily in cattle, buffalo, elk, yaks, and camels. Similarly, *B. suis* and *B. canis*, which infect domestic pigs, rodents, and dogs, are becoming more prevalent as human brucellosis causes (OIE, 2013). Asymptomatically, the disease can cause abortion storms, infertility, and decreased milk production in naive herds, resulting in significant economic losses. Additionally, placentitis, stillbirth, epididymitis, and orchitis are reproductive losses in livestock (Macmillan *et al*., 2006., Mcdermott *et al*., 2013; Abdelbaset *et al*., 2018). Exposure to infected animals or contaminated animal products, consumption of unpasteurized milk and contact with aborted or RFM animals can all result in human infection, presenting clinical signs and symptoms such as persistent intermittent fever, excessive sweating, headaches, tiredness, and chills (Lucero *et al*., 2010).

The disease is now regarded as a re-emerging threat causing public health concern in a number of countries, particularly where animals are the primary source of income including Israel, Kuwait, Saudi Arabia, Brazil, and Colombia, Subsaharan Africa and many other countires (Cutler *et al*., 2005., FAO, 2011). In resource-limited settings such as Ethiopia, brucellosis in animals and humans has been documented in many parts. Occupationally, 11% of animal health workers and 7% of hospital patients are at risk of *Brucella* infection, whereas livestock prevalence varies by geographical region and livestock species, ranging from 3% to nearly 50% (Yilma *et al.*, 2016). Studies to discover the distribution and proportion of *Brucella* species in different natural hosts in the country have so far not enough (Yohannes *et al*., 2013). This is due to a lack of saphosticated laboratory equipments and a low level of laboratory development (Gumi *et al.*, 2013).

Brucellosis has become a major public health concern in pastoral areas because of control strategies of the nation were typically focused on diseases with more severe consequences and the programs involving aspects of brucellosis intervention have generally not been launched (Schelling *et al*., 2004).

Somali region has the most pastoralist communities in Ethiopia, and livestock is their primary source of income. Tschopp *et al*., (2021) reported 8.6% prevalence proportion of livestock brucellosis whereas Teshale *et al*., (2006) reported a prevalence proportion of 1.6% in sheep and 1.7% in goats in Somali region. Seroepidemological studies conducted Lakew *et al*., (2019) and Ibrahim *et al*., (2021) revealed a prevalence of 4.9% and 2.8% in camels and human respectively in Somali region.

A large number of human cases of brucellosis with fever, neurological problems, and other generalized symptoms may be misdiagnosed and treated tentively as malaria or fever of unknown origin in the pastoral community. However, the geographical scope of these studies was limited, and none of them included comparable human brucellosis research in the study area (Megersa *et al*., 2012). Comprehensive studies on brucellosis in different animal species sharing the same ecological zone, as well as zoonotic significance in occupationally linked humans are little in somali region due to insufficient regional laboratory, large livestock herds and flocks, animal migration from and within the country and insufficient veterinary services and husbandry practices. Therefore, understanding human-animal interface of brucellosis, implementing one health approach, further epidemiological study on the circulating *Brucella* species and enhancing herders' KAP related to brucellosis in the study pastoral setting would thus contribute the development of disease control strategies in regional and national level. Hence, the objectives of this study were:-

- To estimate the seroprevalence of brucellosis in camel, goats, sheep and livestock keepers of the study area.
- To identify the associated risk factors with sereprevalence
- To assess the knowledge and exposure risks of the herders’ towards the disease

2. LITERATURE REVIEW

2.1. Brucellosis in Animals

Brucellosis is infectious, re-emerging neglected bacterial disease that affect domestic and wild animals. It is characterized by abortion, still birth and RFM in female and epididymitis, orchitis and sterlity in male (Dabassa, 2013). Primary clinical manifestation of brucellosis among livestock is related reproductive tract. In highly susceptible animals, abortion after five months of pregnancy is the cardinal feature of the disease (Adugna *et al*., 2013).

The disease severely impedes livestock production and causes economic losses at both the household and national levels. As a result, in an extensive production system, the epidemiology, economic consequences, and cost of efficient disease prevention methods in animals and people have not been adequately explored (FAO, 2011).

Prevalence of brucellosis in animals has been reported from various parts in Ethiopia, particularly in small ruminants and camels brucellosis. These prevalence studies are mostly limited to serological surveys and are usually focused on cattle brucellosis, occasionally sheep and goats, and rarely camels. According to the study Kebede *et al*., (2008), herd level prevalence ranged from 2.9% to 45.9%. Similarly, seroprevalence per species was reported from South Eastern Ethiopian pastoral zones of the Somali and Oromia regional state herds (Gumi *et al*., 2013) with 1.4 % prevalence. Similar study from Megersa *et al*., (2011) in the same area showed that anti-*Brucella* antibodies were prevalent(10.6%) in nomadic pastoral societies, where close and frequent contact between man and animal is an unavoidable part of ecology hence, the risk is assumed to be high. In addition, there is little information available for the assessment of herders' KAP towards brucellosis in pastoral settingon (Lokamar *et al.*, 2020).

2.2. Etiology and Characteristics of *Brucella* Organisms

Brucella are facultative intracellular bacteria that include several species from the order *Rhizobials* and the family Alpha *proteobactereacea*. Its rod-shaped (*coccobacillary*) bacteria that are Gram negative, non-motile, and non spore form. They are partially acid fast positive because they don't decolorize 0.5 % acetic acid in Modified Ziehl-Neelsen (MZN) staining techniques. Due to they

retain the carbol fuchsine, the organisms appear as a cluster of red staining *coccobacilli* in MZN stained smears (Quinn *et al*., 2002; OIE, 2012).

There are currently ten *Brucella* species recognized, including six classical species: *B. abortus* (cattle, biovars 1–6, and 9), *B. melitensis* (goats, sheep, biovars 1–3), *B. suis* (pigs, reindeer, and hares, biovars 1–5), *Brucella ovis* (sheep), *Brucella canis* (dogs), and *Brucella neotomae* (desert wood rats) and more recently identified, *Brucella ceti*, *Brucella pinnipedialis* (dolphins/porpoises and seals, respectively), *Brucella microti* (voles), and *Brucella inopinata* (reservoir undetermined). Of these, *B. melitensis* poses the greatest risk of human infection, followed by *B. suis* and *B. abortus*, but several other species have been shown to be virulent for humans (Godfroid *et al*., 2011a). Brucellosis in small ruminants is caused primarily by *B. melitensis* and *B. ovis*, with sporadic cases caused by *B. abortus* and *B. melitensis*. In horses, *B. abortus* together with *Actinomyces Bovis* may be present in poll evil and fistulous withers (Gul and Khan, 2007).

Table 1: Worldwide geographical distribution of *Brucella* species and its biotypes.

Species	Host(s)	Disease	Geographical distribution
B. abortus	Cattle*	Abortion and orchitis	Worldwide(common)
	Sheep, goats and pigs	Sporadic abortion	Worldwide (not common)
	Horses	Associated with bursitis	India, Egypt and East Africa
	Human	Undulant fever	Britain and Germany
B. melitensis	Goats*	Abortion and	Many sheep keeping except
	Human	Malta fever	New Zealand, Australia, and North America in cattle Occasionally
B. suis	Pigs*	Abortion, orchitis, arthritis spondylitis and herd infertility	Worldwide, Western and Central Europe, USA, Argentina and Singapore
B. ovis	Sheep*	Epididymitis in rams and sporadic abortion in ewes	New Zealand, Australia USA, Romania, Czechoslovakia, South Africa and South America
B. canis	Dog*	Abortion, epididymitis,	North America and parts of
	Humans	And permanent infertility in male and Undulant fever	Europe not common
B. neotomae	Desert room rat	Non-pathogenic yet been recovered	USA (Utah)

*Natural host given in capital letters

Sources: (Quinn *et al.*, 2002)

2.3. Pathogenesis

The ability of *Brucella* pathogen to survive and multiply within phagocytic and non-phagocytic cells is its most important feature. *Brucella* does not produce classical virulence factors such as exotoxin, cytolysin, exoensymes plasmid, fimbria, and drug resistant form, rather, lipopolysaccharide (LPS), Type IV Secretion Systems (T4SS) secretion system, and *Brucella* virulence BvrR/BvrS system are major virulence factors at (Fig1), that allow interaction with the host surface. *B. melitensis* inhibits phagosome-lysosome fusion and replicates within compartments containing endoplasmic reticulum components in macrophages (Pizarro-Cerdá *et al*., 1998; Głowacka *et al*., 2018a).

Brucella organisms in ruminants avoid the most effective host defenses by targeting embryonic and trophoblastic tissue. Bacteria grow not only in the phagosome, but also in the cytoplasm and the rough endoplasmic reticulum of these tissue cells. These tissues allow exuberant bacterial growth in the absence of effective intracellular microbicidal mechanisms, resulting in fetal death and abortion (Xavier *et al*., 2010).

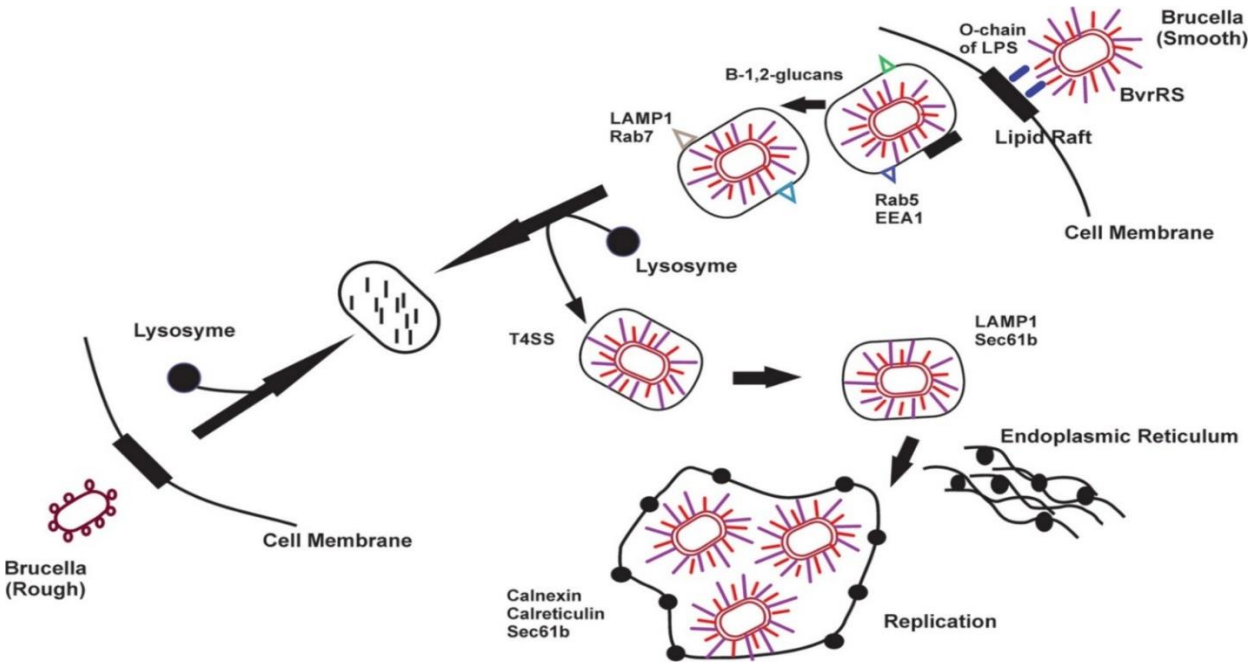

Figure 1*:* Mammalian cell invasion and intracellular trafficking

Source: (Głowacka *et al*., 2018b)

2.4.Clinical Manifestation

The main clinical sign observed in animal in the early stages of the disease is abortion, but other signs due to organism localization may be observed. Among these symptoms are orchitis, epididymitis, hygroma, arthritis, metritis, and subclinical mastitis. Many animals, however, develop a self-limiting infection or become asymptomatic latent carriers and potential excretory. If the female is exposed to the organism at the end of the pregnancy, infection is not established (Poester *et al*., 2010).

The second stage is characterized by either *Brucella* elimination or, more commonly, persistent inflammation of the mammary gland, supramammary, and genital lymph nodes, with constant or intermittent shedding of the organisms in milk and genital secretions. As a result, animals generally abort once during the third trimester of pregnancy, but uterine re-invasion occurs in subsequent pregnancies, with shedding of fluids and fetal membranes (Poester *et al*., 2010).

Females who are give birth in exposed soil and become infected have a lower abortion rate than others. This explains the high rate of abortions in newly infected herds compared to the relatively low rate in enzootic herds. *Brucella* organisms have a strong preference for the udder. Infection in lactating, non-pregnant goats is likely to result in udder colonization and the excretion of *Brucella* organisms in the milk (Poester *et al*., 2010). Females typically have only one abortion, owing to acquired immunity. Abortion with placental retention and the resulting metritis may result in a prolonged calving interval and permanent infertility (Walker, 1999).

People with brucellosis frequently exhibit nonspecific clinical signs and/or symptoms such as malaise, fatigue, fever, muscle and joint pains, and possible spontaneous abortion in pregnant women (Khan *et al*., 2001;WHO, 2006). In human the clinical features are frequently indistinguishable from that of other febrile diseases such as malaria and typhoid fever (WHO, 2006; Zerfu *et al*., 2018). Chronic forms and recurrences of the disease can result in long-term complications such as arthritis, endocarditis, spondylitis, and recurrent fever (Köse *et al*., 2014). In Sub-Saharan Africa, including Ethiopia, where proper diagnostic tools are scarce and information on the causative agents of febrile illness-related symptoms is empiracly treated, febrile illness-related symptoms are frequently misdiagnosed as malaria.

2.5. Diagnosis of Brucellosis

The classical tests for brucellosis diagnosis such as culture and phenotypic characterization, are laborious, time consuming, risk infection, and can produce contradictory results. Similarly, isolation of the agent fails in routine diagnosis. Serological tests are thus commonly used to diagnose *Brucella* in camels and small ruminants at the herd level, but cross reaction with other Gram negative bacteria is a major issue (Gwida *et al*., 2011). In addition, serological tests cannot distinguish between *Brucella* species and thus cannot determine which species has induced host antibodies (Godfroid *et al*., 2010b; Plumb *et al*., 2013).

2.5.1. Serological Tests

The detection of specific antibodies in serum or milk remains the most practical method of brucellosis diagnosis The most efficient and cost-effective method is usually screening all samples using a cheap and rapid test which is sensitive enough to detect a high proportion of infected animals RBPT, Complement fixation test (CFT) and slow agglutination test (SAT) are used for the detection of *Brucella* species. Although RBPT sensitivity meets the requirements for surveillance in field clinics or regional laboratories, it is believed that only the combination of RBPT and CFT/ELISA in infected flocks can obtain accurate individual true seropositivity (Gwida *et al*., 2011c).

The RBPT could be modified for testing of sera in endemic, low prevalence areas to increase the sensitivity of the test. This simple modification is achieved by increasing slightly the number of sera for the test dose from 25 µl to 75 µl, at the same time maintaining the antigen volume at 25 µl. This results in a significant increase in the sensitivity of the test without affecting the specificity (Blasco *et al*., 1994; Erreiraa *et al*., 2003).

Complement fixation test (CFT): When used correctly, it is the most widely used confirmatory test and is recommended by WHO (Garin-Bastuji *et al*., 2006). Regardless of the complexity and heterogeneity of the techniques used in different countries, CFT is based on the detection of specific IgM and IgG1 antibodies that fix complement. It is highly specific, but it has many drawbacks, including being cumbersome, laborious, and requiring highly trained personnel as well as appropriate laboratory facilities. Its specificity is critical for brucellosis control and eradication, but it may test negative when IgG2 antibodies prevent complement fixation (Macmillan, 1990).

Enzyme Linked Immune-Sorbent Assay (ELISA): None of the tests mentioned above can tell the difference between antibodies produced by vaccination and those produced by infection. As a result, various ELISA techniques have been developed to address these issues. Furthermore, ELISA detected *Brucella* carriers who were sero-negative by RBPT, CFT, and SAT (Nielsen *et al.*, 1989). ELISA tests provide excellent sensitivity and specificity while being robust, relatively simple to perform with minimal equipment, and readily available in kit form from a variety of commercial sources.

ELISA technology is now used to diagnose a wide range of animal and human diseases. Although ELISA can be used to test serum from all animal and human species, results may differ between laboratories depending on the exact methodology used. Not all standardization issues have been resolved. Screening tests are typically performed at a single dilution. It should be noted, however, that while ELISA are more sensitive than RBT, they do not always detect infected animals that are RBT positive (WHO, 2006)

Fluorescence Polarization Assay (FPA): is a recently described test used for the serological diagnosis of *Brucella* infection. It is a rapid, homogenous, species independent assay, which was initially developed and validated for the detection of antibodies to *B.abortus* in cattle. FPA has many methodological advantages over the older, more established tests (Gall *et al*., 2000).

2.6. Epidemiology of Brucellosis in Ethiopia

Both the husbandry system and environmental conditions have a significant impact on the spread of *Brucella* infection (Solomon *et al*., 2003). The geographical spread of brucellosis is constantly changing, with new foci emerging or reemerging (Pappas *et al*., 2006). The majority of brucellosis research from highland agro-ecology has focused on urban and pre-urban areas. In Ethiopia, true *Brucella* sero-prevalence was reported to be 5.3% in goats, 2.7% in sheep, and 2.9% in camel (Amenu *et al*., 2010; Lindahl *et al*., 2015). Furthermore, a higher prevalence of 9.0 % livestock was recorded in Afar and 8.6 % in Somali regional state, whereas a prevalence of 48.3 % in Afar and 34.9 % in Somali regional state was recorded in occupationally linked humans (Tschopp *et al*., 2021). Individual animal seroprevalence was found to be relatively low in some intensive farms across the country. Mussie *et al*., (2007) reported a prevalence of 4.63% in Northwestern part of Amhara Regional State.

According to available data, *Brucella* seroprevalence is highest (50%) in indigenous animals in the Borana zone of Southern Ethiopia (Benti and Zewdie, 2015). Seroprevalence per species was reported to be 1.4 % in South Eastern Ethiopian pastoral zones of the Somali and Oromia regional state herds (Gumi, *et al*., 2013). The same study in the area discovered that 10.6% of people had anti-*Brucella* antibodies (Megersa *et al*., 2011c).

In general, brucellosis research in Ethiopia has been limited to serological surveys, with a focus on bovine brucellosis, occasionally sheep and goats, and rarely camels. Attempts to identify *Brucella* species in the country have so far been unsuccessful, and the distribution of their natural hosts has not been thoroughly studied. This is largely due to the laboratory's level of development and a lack of consumables for the tests (Yilma *et al*., 2016).

Several potential risk factors related to production systems, individual host biology, and environmental factors influence brucellosis prevalence (climatic conditions and geography). These factors include species, gender, age, herd size and composition, farm hygiene, rate of contact between infected and susceptible animals, farm biosecurity, and climate (McDermott and Arimi, 2003). Brucellosis was more common in older animals than in younger ones. Pregnant and sexually mature animals are more susceptible to *Brucella* infection and brucellosis than sexually immature animals of either sex (Benti and Zewdie, 2015).

Small ruminants' brucellosis affects sexually matured animals, with male and female reproductive tracts, particularly the pregnant uterus, being preferred sites. This could be due to the fact that the concentrations of sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase with age and sexual maturity. Goats are more susceptible to *Brucella* infection than sheep. This could be because goats are more susceptible to *Brucella* infection. It could also be because goats, unlike sheep, excrete the organism for a long period of time. This reduces the possibility of disease transmission among sheep flocks (Radostits, 2000). The susceptibility of ewes to *B. melitensis* varies by breed. Hence, ewes that produce milk are more receptive (Hirsh, 1999).

Brucella infections are typically transmitted through contact with infected birthing tissues and fluids (for example, placenta, aborted fetuses, fetal fluids, vaginal discharges). The ingestion of contaminated feed or water can infect both camels and small ruminants, and the consequences of the

infection are determined by the virulence of the bacteria, resistance, and reproductive status of the host (Corbel, 2006).

Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, conjunctiva, inhalation, and udder contamination during milking, or by licking an animal's discharge, newborn calf, or retained fetal membrane. The importance of venereal transmission varies by species; for *B. ovis*, *B. Suis*, and *B. canis*, it is the primary route of transmission. *B. abortus and B. melitensis*, on the other hand, can be found in sperm, but venereal transmission of these organisms is uncommon. Above all, free movement and mixing between herds and species can aid in transmission and cross infection (OIE, 2004).

Brucella melitensis is commonly found in camels, sheep, and goats and is highly pathogenic to humans, causing one of the world's most serious zoonosis (OIE, 2004; Benkirane, 2006). Because of abortions, sterility, mastitis, decreased milk production, veterinary attendance, and, most importantly, the prohibition of free animal and animal product trade, Brucellosis causes significant economic losses in livestock production (Hamdy and Amin, 2002).

It is transmitted to humans through occupational contact with infected animals' blood, tissues, or fluids. Consumption of unpasteurized milk and dairy products is the most common method. Human infections can occur through skin breaks caused by handling infected animal tissues. *Brucella* can be transmitted in the laboratory and, most likely, in slaughterhouses via aerosols, contact with laboratory cultures and tissue samples, and accidental injection of live *Brucella* vaccines (WHO, 2006).

Brucella melitensis is typically transmitted in camels through contact with infected animals' placentas, fetuses, fetal fluids, and vaginal discharges. After an abortion or full-term parturition, small ruminants are infectious. Goats typically shed *B. melitensis* in vaginal discharges for at least two to three months, whereas sheep typically shed for three weeks. This organism is also found in milk and sperm; shedding in milk and sperm can be prolonged or lifelong, especially in goats. Children and lambs who nurse from infected dams may excrete *B. melitensis*. The disease spreads due to the movement of infected animals to disease-free herds. The proximity of infected herds to clean herds occurs at water points where a large number of camels congregate (Abuo-Eisha, 2000).

2.7. Status of Human Brucellosis in Ethiopia

Brucellosis is primarily a livestock disease, but it can be transmitted to humans through ingestion, close contact, inhalation, or accidental inoculation. Human brucellosis prevalence varies by region and has been linked to personal and environmental hygiene standards, animal husbandry practices, the species of the causative agent, and local food processing methods. Person-to-person transmission, infection from a contaminated environment, occupational exposure usually caused by direct contact with infected animals, and foodborne transmission are all possible routes of infection for brucellosis.(WHO, 2006).

Person-to-person transmission: This is extremely unusual. Circumstantial evidence suggests that close personal or sexual contact is the route of transmission in a few cases. Transmission via blood donation or tissue transplantation is potentially more significant. Bone marrow transplantation, in particular, is fraught with danger. It is recommended that blood and tissue donors be tested for brucellosis and that positive reactors with a history of recent infection be excluded. Although transmission to brucellosis patients attendants is extremely unlikely, basic precautions should be taken. Similarly, workers in laboratories who process patient samples are at a much higher risk (WHO, 2004;WHO, 2006).

Living in close proximity to livestock, milking, and consuming raw milk and fresh dairy products are the major risks for brucellosis in the pastoral community (Regassa *et al*., 2009a). Human brucellosis research in Ethiopia is limited in comparison to animal, with even less information on risk factors for human infection (Haileselassie *et al*., 2011). For instance, Tolosa *et al*., (2007). revealed that 2 (3.6 %) of 56 cases with fever of unknown origin tested positive for *B. abortus* antibodies.

Regassa *et al*., (2009) conducted a study in traditional pastoral communities using *B. abortus* antigen and found that 34.1% of patients with febrile illness from Borana, 29.4% from Hammer, and 3% from Metema areas tested positive using *Brucella* IgM/IgG Lateral Flow Assay (Table 1). A seroprevalence of 5.3%, 3.8%, and 4.8% by screening sera from 238, 38, and 336 individuals in high-risk groups such as farmers, veterinary professionals, meat inspectors, and artificial insemination technicians in Amhara Regional State, Sidama zone of Southern People Nations, Nationalities State, and Addis Ababa by Kassahun *et al*., (2006). In addition, Animut *et al*., (2009), also examined the prevalence of major causative agents of acute febrile illness in 653 outpatients

from four health centers in Northern Ethiopia. *B. abortus* was found in 6.3%, 3%, and none of the patients in Finoteselam, Quarit, and both Dembecha and Jigjiga, respectively.

Table 2: Seroprevalence report from human brucellosis in different location

Study Area	Prevalence%	Tests employed	References
Amibara district of Afar region	3.3	RBPT+CFT	(Wegi *et al*., 2021)
Babile and Gursum district of Fafen	0.4	RBPT+CFT	(Lakew *et al*., 2019)
Afar region	15	RBPT+CFT	(Zewolda and Wereta, 2012)
Borana	2.6	RBPT+ELISA	(Edao *et al*., 2020)
Adama Tullu	2.15	RBPT+CFT	(Tibesso and Ibrahim, 2014)
Afar and Somali	48.3	ELISA	(Tschopp *et al*., 2021)
Somali region	2.8	ELISA	(Ibrahim *et al*., 2021)
Jimma University Hospital	3.6	RBPT+CFT	(Tolosa *et al*., 2007)
Borana	34.1		(Regassa *et al*., 2009)
Hammer	29.4	RBBT+CFT	
Metema	3		
Tigray	1.2	RBPT+CFT	(Haileselassie *et al*., 2011)
Afar	4.4	RBPT-CFT	(Zerfu *et al*., 2018)

2.8. Status of Camel Brucellosis in Ethiopia

Camel brucellosis is largely understudy in Ethiopia. The first sudy of brucellosis in camle was identified by Domenech, (1977) in Sidama, Harar and Tigray and reported seroprevalence of 4.4%. The current report on camel brucellosis seropositivity in different agro-ecology of revealed a ranges of 0.73–12.2% by RBPT and 0.53–7.6% by CFT Furthermore, Teshome *et al*., (2003) investigated the seroprevalence of brucellosis in arid and semi-arid camel-rearing regions of Ethiopia, reporting 5.7%by RBPT and 4.2% by CFT Table 2. In Borena lowland, Megersa *et al*., (2006) used RBPT and CFT to investigate camel brucellosis in the Borena lowland. *Brucella* antibodies were found in 1.8 % (58/3218) of the camels tested in their study.

Table 3: Seroprevalence of Camel brucellosis from different districts in Ethiopia

Study area	Prevalence (%)	Tests employed	References
Sidama, Harar and Tigray	4.4	RBPT+CFT	(Domenech, 1977)
Afar, Somali and Borana pastoral	4.2	ELISA	(Teshome *et al*., 2003)
Borana lowland, Southern Ethiopia	1.8	RBPT+CFT	(Megersa *et al*., 2006)
Around Dire dawa city, Eastern Ethiopia	1.6	RBPT+CFT	(Mohammed *et a*l., 2011)
Yabello District	2.2	RBPT+CFT	(Megersa *et al*.,2011b)
Afar region, Ethiopia	7.6	RBPT+CFT	(Zewolda and Wereta, 2012)
Southeast Ethiopia	0.9	RBPT+ELISA	(Gumi *et al*., 2013)
Afar region of Northeast Ethiopia	4.1	RBPT+CFT	(Hadush *et al*., 2013)
Afar regional state in Northeastern Ethiopia	5.4	RBPT+CFT	(Wesinew *et al*, 2013)
Jigjiga and Babile	2.43	RBPT+CFT	(Tilahun *et al*., 2013)
Mehoni district, Tigray region	3.37	RBPT+CFT	(Habtamu *et al*., 2015)
Akaki abattoir	4.1	RBPT+CFT	(Abebe *et al*., 2017)
Afar region	4.1	RBPT+CFT	(Gizaw *et al*., 2017)
Jigjiga and Gursum districts of Fafen Zone	4.9	RBPT+CFT	(Lakew *et al*., 2019)
Adadle district of Somali region	0.6	ELISA	(Ibrahim *et a*l., 2021)
Pastoral Afar and Somali	7.5	ELISA	(Tschopp *et al*., 2021)
Amibara district of Afar region	3.2	RBPT+CFT	(Wegi *et al*., 2021)
Dire dawa , Eastern Ethiopia	2	RBPT+CFT	(Waktole *et al*., 2022)

2.9. Status of Small Ruminant Brucellosis in Ethiopia

Teshale *et al*., (2006*)* tested sera from 2000 sheep and goats in pastoral regions of Ethiopia and found 1.9% positive using RBPT and 9.7% positive using I-ELISA. In contrast, a cross-sectional study of 1568 serum samples from sheep and goats in Afar's pastoral region revealed 9.4% positive using RBPT and 4.8% positive using CFT (Ashenafi, *et al*., 2007). Wubishet *et al*., (2018) discovered an overall seroprevalence of 8.5% RBPT and 2.3 percent cELISA in Borana low land.

Table 4: Seroprevalence of Small Ruminant Brucellosis in Different Locations in Ethiopia

Study area	Prevalence (%)		Tests Employed	References
	Sheep	Goats		
Borana	1.5	4.0	ELISA	(Edao *et al*., 2020)
Afar	3.2	5.2	RBPT+CFT	(Ashenafi, Teshale, Agga, *et al*., 2007)
Adadle, Somali region	8.3	9.7	ELISA	(Tschopp *et al*., 2021)
Somali Region	0.9	2.7	RBPT+CFT	(Lakew *et al*., 2019)
Afar and Somali region	5.6	13.2	RBPT+ELISA	(Teshale et al., 2006)
Bahir Dar, North East Ethiopia	0	0.4	RBPT+CFT	(Ferede *et al*., 2011)
Yabello District of Borana Zone	6.1	9.2	ELISA	(Wakene *et al., 2017)*
Pastoral area of Somali and	0.48	3.09	RBPT+CFT	(Tsehay *et al*., 2014)
Somali region	1	1.57	RBPT+CFT	(Mohammed *et al*., 2017)
Borana pastoral area	-	17.36	ELISA	(Teshome et al., 2022)
Boku live sheep export	0.6	-	RBPT+CFT	(Girmay *et al*., 2013)
South Omo zone	-	4.2	RBPT+CFT	(Tigist Ashagrie, 2011)
Yabello district	1.17	1.88	RBPT+CFT	(Dabassa, 2013)
Yabello District	-	1.9	RBPT+CFT	(Megersa *et al*., 2011)
Yabello district	6	9.2	RBPT+ELISA	(Wubishet *et al*., 2018)

Effective brucellosis prevention and control is dependent on (KAP) of pastoral communities where the diseas is most prevalent due to close interaction between pastoralists and their animals, and animal populations in such settings are typically ill defined or unknown (Obonyo, 2015). Several assessments of the KAP of animal attendants in Ethiopia have revealed a low KAP towards brucellosis and other zoonotic diseases in general (Legesse *et al*., 2018). A descriptive pastoral community-based cross-sectional study was conducted to determine whether abortion is a major problem in their livestock, community members' knowledge of brucellosis as a cause of abortion in animals and its zoonotic significance, mode of transmission from animals to humans, and risk practices for acquiring *Brucella* infection in humans. Almost all study participants stated that abortion in livestock, particularly goats and sheep, is a major issue in the study area. (Legesse *et al*.,

2018). According to Kenea and Megersa, (2021), majority of the pastoralists (72.7%)) lacked basic information about brucellosis, which could increase the risk of transmission.

2.10. Prevention and Control

Vaccination is one of the most effective methods for preventing and controlling livestock brucellosis. Both live vaccines, such as *B. abortus S-19, B. melitensis Rev1, B. suis S-2, rough B. melitensis strain M111*, and *B. abortus strain RB51*, and killed vaccines, such as *B. abortus 45/20* and *B. melitensis H.38,* are available in various parts of the world. The use of the *RB51* attenuated live vaccine to control brucellosis in cattle has recently gained popularity (Cheville *et al*., 1996). However, in our country Ethiopia, due to lack of vaccine available, it is only possible to implement measures to reduce the risk of infection through personal hygiene, the adoption of safe working practices, and the prevention of contamination of water sources and pasture. Furthermore, theimplementation of national brucellosis eardicatioln program (Yohannes *et al*., 2013).

2.10.1. One health approach

The implementation of one health approach and the establishment of national veterinary extension services in the country are crucial for raising awareness about brucellosis, its impact on livestock production and zoonotic risks, as well as providing a feasable prevention measure. This would aid in uniting both community animal producers in the fight to control and eliminate brucellosis (Yohannes *et al*., 2013). This work could help raise disease awareness and improve laboratory services for diagnosing brucellosis and other zoonotic diseases. So far, several achievements have been recorded in Ethiopia, including the extension of one health scheme to regional governments, joint disease surveillance and outbreak investigation activities, joint vaccination activities against zoonotic diseases, zoonotic disease prioritization, and the development of control and prevention strategic documents for various prioritized zoonotic diseases. However, community and responsible bodies' awareness of one health principles and importance is limited (Erkyihun *et al*., 2022).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in three purposively selected districts of Somali region namely, Gurabaqaqsa, Guradhamole and Dolo Ado of Liban Zone. Liban zone borders Kenya to the South, Somalia to the Southeast, and Oromia region to the Northwest and Afder zone to the Northeast. So far the zone was only four districts, Dolo Ado, Filtu and Boqolmayo, Deka suftu but in 2015, three more districts were added (Gurabaqasa, Guradhamole and Qundhi). Majority of the communities are pastoralists (90%), keeping livestock for their daily livelihood and sources income. The climate is typically arid to semi-arid, and many places experience regular water and fodder shortages, forcing pastoralists to seasonal migrations with their animals. Somali region state district added these two district from Afdher zone to Liban zone (SRBoFED, 2014).

The zone's altitude ranges from 250 to 1500 meters above sea level and is located between 6°00′N 43°45′E. The average annual rainfall in the area ranges between 600 and 700 mm. The main rainy season, known as "Gu," lasts from March to May, followed by the short dry season, known as "Xagaa," which lasts from June to August. The short rainy season "Dayr" occurs between September and November, and the long dry season "Jilaal" occurs between December and March. In 2000, the majority of people in the Liban Zone (70.93 percent) were pastoralists, while 25-30 percent were agro-pastoralists and 40-50 percent practiced riverine cultivation (Ibrahim *et al*., 2021). The zone has a livestock population of 503,871 cattle, 1,134,856 sheep, 1,365,265 goats, and 290,649 camels (Gebre-Mariam, 2016). Currently, the zone and the state in general is facing severe drought which is causing high livestock mortality and morbidity as well massive migration of pastoral community to water sources and pastures.

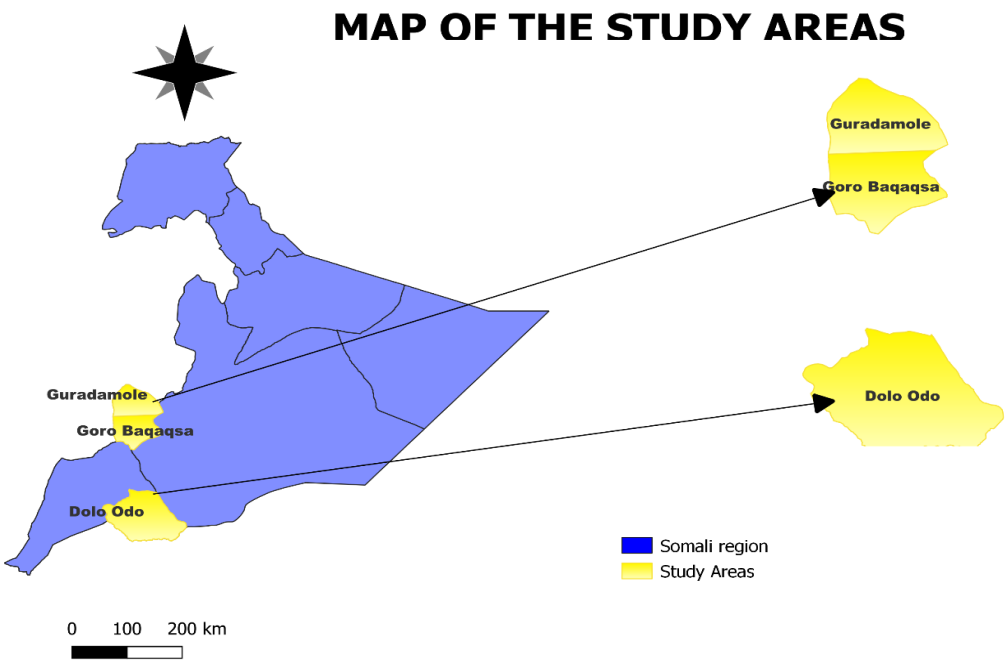

Figure 2: Map of study areas developed from Ethiopian shape files using QGIS

3.2. Study Design and Study Population

A cross sectional study was undertaken from December, 2021 to April, 2022 to determine brucellosis: seroprevalence, knowledge and exposure risks of herders in Somali region, Ethiopia.

Livestock: The targeted population of camel, sheep and goats where composed of blackhead Somali sheep, long ear Somali goats and local camel breed. Putative Biological and environmental factors believed to be associated with epidemiology of brucellosis were recorded. These included districts, species, sex, age, herd size, migration, and numbers of parity, herd history of abortion and RFM, and physiological status of the animals.

Human: Several known high risk behaviors were common among self-reported practices of pastoral community in Liban zone, Somali region. Such behaviors were consumption of unpasteurized milk and not wearing protective clothes when dealing with animal and products or using bare hands when assisting delivery animals or with aborted materials. The selected Peasant Association (PAs) or Kebeles in the study areas were sampled by a hired professional nurse from the human health posts

for the collection of human samples. A consent form indicating the purpose of the study was filled and signed before sample collection. Blood samples were collected from voluntary livestock owner and attendants. Potential human risk factors for brucellosis such as age, gender, had fever or joint pain, presence of more than three species of animals, disposal of aborted or RFM and whether they heard of brucellosis were considered.

Exclusion Criteria: During sampling, volunteer individual were further asked about their knowledge, attitude and practice towards brucellosis, therefore individuals younger than 18 year where excluded from the study for their responses may create bias.

3.3. Sampling Method and Sample Size Determination

A multistage sampling combined with the convenient sampling strategy were employed. Three districts were purposively selected due to their livestock population and proximity to road. A Peasants Association (PA) or a village is the smallest administrative unit in the study district. The total number of PAs within the three districts were listed and used as sampling frame. Therefore, six PAs were randomly selected and used as a cluster units. For the purpose of this study, a households (HHs) was a group of people who use a common cooking area. Subsequently, HHs keeping three livestock where given priority to be sampled, if not at least two species from the same household. Within each randomly selected household, all people were enrolled if they had provided informed consent to participate in the study.

The number of each animal to be sampled per PA was estimated taking the dynamic nature of pastoral herds (high herd mobility), presence of three species, willingness of the herd owners to cooperate, availability of the herds during the visit into consideration. The average livestock holding per household was estimated to be 25 goats, 20 camel and 5 sheep. As a result the expected prevalence of 7.5% in camel Ibrahim *et al*., (2021) and 9.7% Teshale *et al*., (2006) in sheep and goats with 5% desired absolute precision and 95% confidence interval was assumed to calculate the desired sample size in camel, sheep and goat. Accordingly, a minimum sample size was 107 camel, 134 sheep and goats from the three districts. Hence this minimum target was reached by serum sampling, a total of 450 camel, and 300 sheep and goats was collected from the target PAs. Similarly, with the expected prevalence of human 16.5% Ahmed *et al*., (2009) with 5% desired

absolute precision at 95% confidence interval, a total of 250 samples were collected from occupationally linked individuals.

For questionnaire survey, sample size was calculated using the formula given by Arsham, (2002) N=0.25/SE2, where: N = sample size, SE (standard error) = 5%. Thus, at total of 100 participants were interviewed from their knowledge, attitude, and practice (KAP) towards brucellosis.

3.4. Sample Collection and Laboratory Analysis

3.4.1. Blood sample collection

To minimize error, a bar code system was developed for both human and animals samples. The code consists of abbreviated region, district, and Kebeles (SLGBB0001). Care was given to ensure that a matching label was fixed to the vacutainer tube after blood collection. After properly restrained and disinfected with 70% alcohol, 10ml and 4-5ml of blood were drawn from the jugular vein of the camel, sheep and goats respectively. The blood from each animal were labeled and left tilted over night at room temperature to allow for clotting. Sera was harvested from the clots by siphoning them in to sterile cryovials. In human sample approximately 5ml of blood was drawn by a qualified nurse at the Kebeles via venipuncture of the medium cuboidal vein using plain EDTA vacutainer tube. The sera samples were then transported to Jigjiga regional veterinary diagnostic and research laboratory with an ice box and stored at -20 ^{0}C for further processing.

3.4.2. Serological test

RBPT: Primary screening of serum samples for *Brucella* antibody were performed at Jigjiga Regional Veterinary Diagnostic and Research Laboratory according to the standard procedure described by (Klaus Nielsen, 2002). The results were read by examining the degree of agglutination in good light source and when necessary using magnifying glass. Any visible agglutination were considered positive. Based on the concentration of antibody, there was weak, moderate and strong agglutinations. For interpretation of the results, both positive and negative control sera were used as recommended by OIE, (2004). For sheep and goats, in order to improve the sensitivity of RBPT as previously recommended, one volume of antigen and three volumes of serum (e.g. 25ul with 75ul) was used for the test. The antigen and test serum were mixed thoroughly with a plastic applicator, shaken for 4 min, and the result (presence of agglutination or not) was read immediately (OIE, 2004).

Competitive ELISA. All RBT positive sera were further tested at Jigjiga Veterinary Diagnostic and Research Laboratory. According the manufacturer, camel and small ruminant sera (sheep, goat) were analyzed using a commercial cELISA (Abbexa Ltd., Cambridge Science Park, and Cambridge, CB4 0EY, UK). Human samples were tested using an Ig G ELISA (Abbexa LTD, Cambridge, UK). For camel samples, negative result, if the OD value < CUT OFF, the sample is Camel *Brucella* negative and positive result, if the OD value ≥ CUT OFF, the sample is Camel *Brucella* positive. For small ruminant samples, if PI ≤ 70%, the test samples are considered negative and if PI > 70%, the test samples are considered positive. Ultimately, human sample, If OD of Samples < CUT OFF, the test samples are considered negative and If OD of Samples ≥ CUT OFF, the test samples are considered positive

Case definition: An animal or human case was considered positive, if it tested seropositive on both RBPT and cELISA in serial interpretation. Similarly, a herd or flock was considered seropositive when at least one animal in a herd or flock tested positive. Since there is no history of vaccination against brucellosis in Ethiopia, seropositivity observed in this study was considered due to natural infection of *Brucella*.

3.4.3. Questionnaire survey

Questionnaire was done using semi-structured and structured questionnaire (Annex iii). The questionnaire focused on the (KAP) towards brucellosis. The collected data were on potential risk factors associated with brucellosis including, clinical signs, transmission routes, information, species affected and others. Furthermore, consumption practice of meat and milk, milking, handling of aborted fetus materials or RFM of the community were considered.

3.5. Ethical Consideration and Clearance

The study received Ethical clearance from both Addis Ababa University College of Veterinary Medicine (AAU-CVM) with certificate Ref: VM/ERC/21/02/142022) and Somali Region Health Bureau with (Ref: SRHB-18-7738/2022). Additionally, verbal consent was obtained from the owner of the animal. Full cooperation and voluntary participation of all participants was obtained by assuring them the confidentiality of their involvement.

3.6. Data Analysis

The data from the field and laboratory were entered into Microsoft Excel and analyzed using both R studio version 4.0 and STATA version 14 (Stata Corp, College Station, Texas). In addition to descriptive analysis, association of *Brucella* seropositivity with risk factors was considered using logistic regression. Univariable logistic regression model was used to identify potential risk factors associated with *Brucella* infection, at herd or flock level. Variables with a *P*-value less than or equal to 0.2 (in univariable analysis) were included in the multivariable logistic model. However, in this study, the number of outcomes of interest were less than 10% of the total sample size of human sample, in addition to zero outcomes in some levels. Thus, Firth's biased reduced logistic regression model was used to measure the association of potential risk factors with *Brucella* seropositivity (Puhr *et al*., 2017). Kruskal-Wallis H test (a rank-based nonparametric test) was used to analyze the effects of socio-demographic factors on knowledge, attitude and practice (KAP) of respondents towards Brucellosis.

4. RESULTS

4.1. Seroprevalence of Livestock and Human Brucellosis and Associated Risk factors

Out of 1000 animal and human serum collected, 99 were sheep, 201 were goats, 450 were camels and 250 were human. An overall seroprevalence of 3.7% (95% Cl; 2.5-5.4) was recorded from livestock whereas, 2.0% was recorded from humans using cELISA. The highest seroprevalence of (6.5%; 95% Cl, 3.5-10.8) was observed in goats compared to camels (2.9%; 95% Cl, 1.5-4.9) and sheep sheep (2.0 %, 95%CI, 0.2-7.1). The seropositivity was higher in females (5.2%) compared to the male (1.4%) animals. Regarding to districts, the overall prevalence of livestock range from 3.2% to 4%. At Table 5, shows the seroprevalence of occupationally linked individuals was higher in old aged people (8.3%; 95% Cl=0.7-20.0) compared to other age categories.

Statistical significance was observed between sex, herd size and herd history of RFM ($P<0.05$) and seropositivity of *Brucella*. Among species, camel had less risk of seropositive (OR: 1.1; P=0.069) when compared with the other species. The odds of animals that are kept in large herd were 3 times more likely to be seropositive than those from small herd size. Species showed marginal significant and the seroprevalence was about more than 2 times fold in goats than camel, sheep. Comparing age category, old animals are twice more likely to be seropositive than young animals ones. The proportion of animals with history of retained fetal membrane showed significantly higher (OR: 15.7; 95% Cl=4.6-54.3) in seropositive animals compared to sero-negative animals.

Table 5: Univariable Logistic Regression Analysis for the Prevalence of Livestock Brucellosis

Variables	Category	Samples	Positive (%)	OR(95% Cl)	P-value
Districts	Gura-baqaqsa	250	10(4)	0.7(0.3-2.0)	0.57
	Gura-dhamole	250	10(4)	0.5(0.2-1.5)	0.24
	Dolo Ado	250	8(3.2)	Ref	
Species	Goats	201	13(6.5)	4.4(0.9-22.1)	0.07
	Sheep	99	2(2.0)	Ref	
	Camel	450	13(2.9)	1.1(0.3-6.7)	0.66
Sex	Female	462	24(5.2)	0.2(0.1-0.6)	0.01*
	Male	288	4(1.4)	Ref	
Age	Young	377	9(2.4)	Ref	
	Old	373	19(5.1)	2(0.8-4.3)	0.14
Herd-size	<50	318	7(2.2)	Ref	
	≥50	432	21(4.9)	3.3(1.3-8.3)	0.01*
Migration	Yes	448	17(3.8)	0.6(0.3-1.5)	0.30
	No	302	11(3.6)	Ref	
No. of Parity	Null	217	7(3.2)	Ref	
	≤3	229	16(7)	0.7(0.2-2.2)	0.49
	>3	16	1(6.3)	Ref	
Abortion	Yes	84	10(11.9)	1.1(0.3-2.0)	0.87
	No	378	14(3.7)	Ref	
RFM	Yes	31	12(27.9)	15.7(4.6-54.3)	0.00*
	No	407	12(2.9)	Ref	
PS	Dry	284	10(3.5)	Ref	
	Pregnant	92	10(10.9)	1.0(0.5-2.1)	0.89
	Lactating	86	4(4.7)	Ref	

PS= Physiological Status, RFM=Retained Fetal Membrane, Ref= category references

The variables with P<0.05 from univariable logistic regression analyses were included in the multivariable logistic model. Age, Herd size and RFM were taken to the final model. Multivariable

logistic regression, animals with the history of RFM are 16.7 times more likely exposed *Brucella* infection OR=16.67, 95% Cl =5.42-51.2 than those with no previous history of RFM (Table 6).

Table 6: Multivariable Logistic Regression Analysis for Livestock Brucellosis

Variable	Category	Samples	Positive (%)	Adjusted OR(95% Cl)	P-value
Age	Young	377	9(2.4)	Ref	
	Old	373	19(5.1)	0.7(0.2-2.3)	0.62
Herd size	<50 animals	318	7(2.2)	Ref	
	>50 animals	432	21(4.9)	2.1(0.7-5.3)	0.14
RFM	Yes	31	12(38.7)	16.7(5.42-51.2)	0.00*
	No	407	12(2.9)	Ref	

The seroprevalence of brucellosis in occupationally linked household members and its association with demographic factors in the three districts were found to be 2.8% (n = 2) in Gurabaqaqsa, 1.3% (n = 1) in Guradhamole, and 2% (n = 2) in Dolo Ado districts. Relatively, higher seroprevalence was observed in male individuals 3.4% (n = 3) compared to females 1.2% (n = 2). Among the variables subjected to univariable Firth's logistic regression analysis, gender, presence of fever or joint pain and handling aborted or RFM were significantly associated with increased risk of brucellosis in humans (P< 0.05). Individuals having history of fever and joint pain were 5.7 times more likely to be seropositive for *Brucella* infection than those who had no history of fever or joint. Similarly, old aged individuals had four folds higher odds of *Brucella* seropositivity than others (OR = 3.9; 95% CI = 0.8-20.3). Handling and disposing aborted materials and RFM had increased the likelihood of seropositivity by 26 times more (OR = 26, 95% CI = 4.6-17).

Table 7: Univariable Firths' Logistic Regression Analysis of Human Brucellosis

Variable	Categories	Samples	Positive (%)	OR (95% CI)	P value
Gender	Male	89	3(3.4)	Ref	
	Female	161	2(1.2)	7.0(1-68)	0.03*
Age	Young	37	1(2.7)	Ref	
	Adult	177	1(0.6)	Ref	
	Old	36	3(8.3)	3.9(0.7-2)	0.10
Own Goats	yes	182	4(2.2)	1.51(0.2-13.7)	0.72
	No	68	1(1.5)	Ref	
Fever/ joint	yes	196	3(1.5)	5.71(0.9-35.1)	0.04*
pain	No	54	2(3.7)	Ref	
Ass.	yes	54	4(7.4)	1.13(4.1-17.4)	0.91
Calving	No	196	1(0.5)	Ref	
Milking	Yes	160	4(2.5)	2.3(0.3-2)	0.46
	No	90	1(1.1)	Ref	
Dispose	yes	13	3(23)	26(4.8-17)	0.00*
RFM	No	237	2(0.8)	Ref	
Heard of	yes	70	1(1.4)	Ref	
brucellosis	No	190	4(2.1)	3.06 (0.3-28.5)	0.19

Explanatory variables with P<0.2 in univariable Firth's logistic regression analysis where subjected to multivariable Firth's logistic regression analysis model (Table 8). Therefore, analysis showed that age and dispose aborted fetus or RFM were statistically significant (P<0.05). Moreover, female participants were 7.3 times more likely of contracting brucellosis when compared with counterpart and also showed statistically significant association (P=0.043; OR; 95% Cl=7.3(1.1-50.5).

Table 8: Multivariable Firth's logistic Regression Analysis of Human Brucellosis

Variable	Category	Sample	Positive %	OR(95% Cl)	P-value
Gender	Male	89	3(3.4)	Ref	
	Female	161	2(1.2)	7.3(1.1-50.5)	0.043*
Age	18-35	37	1(2.7)	Ref	
	35-60	177	1(0.6)	Ref	
	Above 60	36	3(0.6)	2.9(0.4-2.3)	0.214
Fever/Joint pain	Yes	196	3(1.5)	4.0(0.9-18.4)	0.109
	No	54	2(2.7)	Ref	
Dispose aborted materials or RFM	Yes	13	3(23)	9.8(5.1-18.5)	0.002*
	No	237	2(0.8)	Ref	
Heard of brucellosis	Yes	70	1(1.4)	Ref	
	No	180	42.0	2.6(0.4-2)	0.303

4.2. Results of the knowledge, attitude and Practice (KAP) towards Brucellosis

Table 9 illustrates knowledge questions asked about brucellosis, 48% of the respondents heard brucellosis, of those who heard, nearly three quarter answered more five questions correctly. Further, participants who heard about brucellosis (53.3%) and 41.7% mentioned community and health worker as their information sources respectively. Similarly, about 35.4% of the respondents replied that brucellosis is an animal health problem in their area. Regarding the species affected by brucellosis, they responded that camel 12 (25%), cattle 10(20.8%), goats 19(39.6%), and sheep 7(12.5%) were common species affecting the disease. The clinical symptoms mentioned for animals were RFM (27.0%) and abortion (10.4%). From the respondents, 16.7% of them reported that contaminated feed and water are the means transmission of brucellosis to animals, whereas, about 8.3%, and 2.1% of the respondents mentioned raw milk and contact with infected animal as means of transmission to humans. When asked about sources of infection, they belief that contact (12.5%) and mating (6.25%) are main sources of infections. Even though, nearly half of respondents heard about brucellosis, very few respondents (14.6%) have mentioned that the disease has zoonotic importance and can causes fever (10.5%) and joint pain (4.17%) in human.

Table 9: Knowledge of Response towards Brucellosis

Knowledge of brucellosis	**Responses (%)**	
	Yes	**No**
Have you ever heard about brucellosis?	48	58
Is brucellosis an animal health problem in this area?	35.4	66.6
Which livestock species are affected by brucellosis?	18.9	81.1
Clinical signs of brucellosis in camel, sheep and goats?	18.0	82
Does it transmit from animal to animal?	35.4	64.6
Mode of transmission to animals	17	83
Does it transmit from animal to human?	16	84
Mode of transmission to human	7	93
Clinical signs in human	5	95
What is treatment means in animals?	7	93

Regarding positive attitude toward consumption of raw milk, low number (7%) of participants mentioned boiled milk prevent brucellosis, and considerable number (29.1%) mentioned milk from aborted animals has health problem. In contrast to the habit of consuming cooked meat consumption, they showed that 4% positive attitude towards the disease. In relation to the washing hands after contact with animals, high number of respondents (71.1%) specified yes. Concerning attitude towards disposing of aborted material or RFM at the field has health problem, few number (8%) mentioned positive attitude in (Table 10).

Table 10: Attitudes of the Herders towards Brucellosis

Attitude of the respondents	**Responses (%)**	
Do you think?	**Yes**	**No**
Boiling milk prevent brucellosis?	7	93
Cooking meat before eating prevents brucellosis?	4	96
Washing hands after handling after contacts with animals prevent brucellosis?	71.1	28.9
You need further information about brucellosis?	71	29
Animal brucellosis is treatable?	72.1	27.9
Human brucellosis is treatable?	39.1	60.9
Drinking milk from aborted animals has health problem?	29.1	70.9
Consumption of raw organs (meat, liver, kidney, meat, tongue) has health problem?	85	15
Disposing aborted material in the field has problem?	8	92

Most of the respondents from the study areas, indicated that they performed several exposure (risky) practices frequently such as, consume of raw milk (90%), milk from aborted animals (85%) and fresh urine or blood (52%) (Table 11). From the preventative practices, the habit of separating animals during parturition was lower (1%), compared to the practice of separating aborted animals (3%).

Table 11: Practices towards Brucellosis

Practices towards Brucellosis	**Frequency (%)**	
Do you and your family	**Yes**	**No**
Consume raw milk?	90	10
Drink milk from aborted animals?	85	15
Consume fresh urine or blood?	52	48
Assist delivery with bare hand?	94	6
Remove placenta by bare hand?	99	1
Handle aborted fetus with bare hand?	79	21
Preventive Practice		
Boil milk for consumption?	6	94
Separate animal during parturition?	1	99
Separate aborted animals?	3	97
Properly dispose fetal membrane?	11	79
Wash hand with soap after delivery?	30	70

From Kruskal Wallis analysis, the result of KAP varied with socio-demographic variables such districts, gender, age, family size and educational. The statistical analysis showed that the mean score of attitude compared to knowledge and practice. The association of socio-demographic factor with the KAP, factors such as districts and education of the participants were statistically significant ($P<0.05$) (Table12). Associating knowledge, individuals with primary education showed good knowledge compared to the other categories of education. However, individuals with secondary education and above showed higher positive attitude toward the disease. Whereas respondents above 60 years of old showed good practice compared to the other age categories.

Table 12: Kruskal-Wallis H test of Factors Affecting Knowledge (n=12), Attitude (n=9) and Practices (n= 16) towards Brucellosis

Variable	Category	Respondents	K Score	A Score	P Score	K P-value	A P-value	P P-value
Districts	G/baqaqsa	30	0.2	3.4	3.7	0.00*	0.001*	0.01*
	G/dhamole	30	1.5	4.1	4.2			
	Dolo Ado	40	0.1	5.1	3.2			
Gender	Male	59	0.1	5.1	3.5	0.26	0.163	0.55
	Female	41	0.2	5.2	3.7			
Age	18-35	40	0	5.2	3.5	0.98	0.832	0.49
	35-60	38	0.1	5	3.6			
	>60	22	0.6	5.1	4.0			
Family	0-5	37	0.5	4.5	3.7	0.19	0.165	0.66
Size	5-9	43	1	5.5	3.7			
	Above 9	20	3.6	5	3.5			
Education	illiterate	73	1.1	3.9	3.3	0.05	0.00*	0.00*
	Primary	16	1.9	4.3	3.4			
	≥Secondary	11	0.7	6.3	3.5			

Whereas, K= Knowledge, A= Attitude P=Practice

Analysis of KAP, showed that several potential risk factors asked by participants related to attitude. In relation to knowledge and practice, herders reflected positive attitude towards question in Fig 3. Although knowledge of the participants from the study unfavorable (<50%) still, participants from Gura-baqaqsa, old aged and primary school revealed they have good knowledge compared to the other categories. Even though, the respondents showed favorable (>50%) answers, individuals with secondary and above showed higher than the other socio-demographic of the participants.

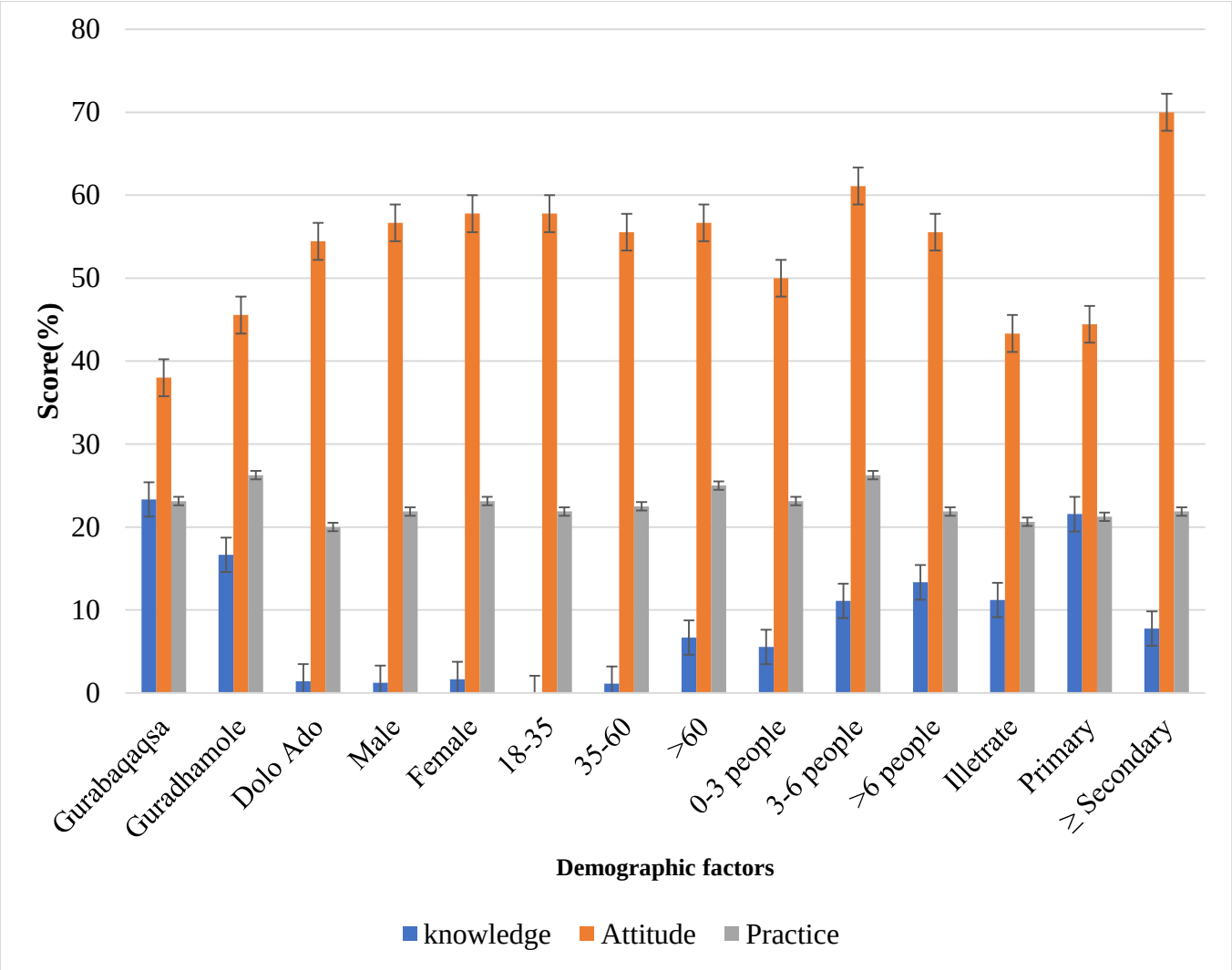

Figure 3: Average KAP score of respondents based on the correct answers

5. DISCUSSION

Brucellosis is prevalent among people and livestock in the Somali pastoral region of Ethiopia. The overall seroprevalence of livestock brucellosis in this study was 3.7% (95% Cl; 2.5-5.4). The observed prevalence could be attributed to seasonal migration, inadequate veterinary services, animal migration and lack of awareness among owners. The current study is lower than 9.7% found by Teshale *et al*., 2006 from Somalia and Afar region. The difference is due to variations in husbandry practice, serological tests, and sample size employed.

According to a recent study, goats have a higher seroprevalence of brucellosis (6.5%) than 2% in sheep. The higher prevalence is due in part to goats' greater susceptibility to *Brucella* infection than sheep, and in part because sheep, unlike goats, do not excrete the *Brucella* organisms for longer periods of time. This reduces the possibility of disease spread among sheep flocks (Radostits, 1994). This is in line with the report of (Ashenafi, *et al*., 2007. However, higher prevalence was reported by 9.6% Gumi *et al*.,(2013) from Southeast Ethiopian pastoral livestock and 27.7% Al-Majali, (2005) from Jordan; such contrasting results are primarily due to differences in husbandry practices, country diversity, and sensitivity of serological tests used.

Females and older animals had higher seroprevalence than males and younger animals. This is consistent with Radostits *et al.,* (2000). It has already been demonstrated that sexually mature animals are more susceptible to brucellosis. Young animals are frequently resistant, but it should be noted that latent infections can occur, and such animals may pose a risk when mature (Corbel *et al*., 2011).

The observed higher seropositivity in the large herds agrees with previous study findings of Ferede *et al*., (2011) and Megersa *et al.,* (2011). The similarlity could be due to increase in stocking density and frequent contacts among animals in large herds, one of the determinants for exposure to *Brucella* infection especially during the time of abortion or calving (Radostits *et al.,* 2007).

Seroprevalence of camel brucellosis was 2.9% (95% CI=0.013-0.04), this result is similar with 2.43% of Tolosa *et al*., (2008), 3.2% of Wegi *et al*., (2021) and 2% of Waktole *et al*., (2022.) However, this result is lower than 5.4% of Zewolda and Wereta, (2012) and 7.6% of Bekele *et al*., (2013). The observed variations in the seroprevalence might be due to differences in management

and husbandry practices, the virulence of the organism, coverage and quality of veterinary services, degree of awareness, the extent of susceptibility of the animals, and unrestricted movement of among pastoralist people.

The increased incidence of brucellosis in pregnant and sexually matured animals is associated with the increased production of erythritol sugar at the stage of life, which enhances the multiplication of pathogen (Gizaw *et al*., 2017). In this study, higher *Brucella* seropositivity was found in adult and old camel than in younger animals, as it a disease of sexually matured and pregnant animals. In univariate and multivariable logistics regression analysis revealed that putative biological factors herd history of RFM showed association seropositivity of *Brucella* (P<0.05). These study coincided with study conducted in Yabello district by Wubishet *et al*., (2018). This could due to unknown cause abortion with RFM outbreak in 2020/2021 in study areas.

Human brucellosis is a prevalent disease in pastoral areas of Ethiopia. The overall prevalence in this study was 2.0% (n=5/250; Cl= 0.0-0.04)) in occupationally linked individuals in a combined RBPT and cELISA, indicating the importance of disease in pastoral areas for public health. This finding is consistent with the findings of Edao *et al*., (2020), who reported 2.6% in Borana and Ibrahim *et al*., 2021 who reported 2.8% in Somali region. However, the recent data is lower than the study of Tschopp *et al*., (2021) who reported 48.3% in Afar and 34.9% in Somalia region. The difference in seropositivity could be associated with the degree of brucellosis endemicity in the livestock population, degree of exposure, sample size setting of the study population, difference in location, and variability related to diagnostic test and method applied as well as the different time period conducted the study.

In pastoral communities, milking and cleaning animals of animals at households is mainly carried out by female, resulting in close proximity and a higher risk of contracting the *Brucella* pathogen. The recent study found that females have a 7.3 times higher prevalence than males, which was statistically significant (P<0.05) with brucellosis seropositivity and did not match with the report of Zerfu *et al*., (2018) in Afar.

In comparison to the other age groups, older participants (>60 years) were more prevalent (8.3%), which is due to *Brucell*a seropositivity increasing with age, the longer you live, the higher chance you get once exposed to the agent and multiparty as they get older (Tay *et al*., 2015). As a result,

elderly people have 2.9 times the odds of being seropositive (OR; 2.9; 95% Cl; 0.7-20) than young and adult people. Tschopp *et al*., (2021), reported similar findings from Afar and Somali Regional States. This study, however, found no significant association in the age category (P>0.05), whereas Teshome *et al*., (2022) found a significant association.

Because *Brucella* are known to prefer the reproductive organs of female animals, particularly the placenta and aborted tissue, it is reasonable to assume that improper disposal will increase the risk of transmission (Osoro *et al*., 2015). As a result, our study found it to be statistically significant (P<0.05). Individuals who did not properly dispose of aborted materials or RFM had a 26-fold higher risk than those who did. Our study fairly disagree the report from Edao *et al*., (2020. The difference could be due to a difference in the number of respondents, the level of awareness at the study area

Conserning herders' knowledge of brucellosis, our study found that 48% of participants had heard of brucellosis. In comparison, the recent study differ from those of Kenea and Megersa, (2021); Njenga *et al*., (2020) who reported that 72% and 84% of their respondents, respectively, had heard of brucellosis. The different could be Locations, number of respondents, and level of awareness (community engagement) in the study areas. Among those who had heard about the disease, (53%) mentioned community and (41.7 %) health workers as sources of information. This is comparable to Obonyo, Mark and Gufu, (2015).

clinical signs of brucellosis in animals, a quarter of respondents mentioned RFM (27%) and abortion (10%) as common clinical signs. This finding is consistent with Njenga *et al*., (2020); Lindahl *et al*., (2015) and Kenea and Megersa, (2021), who mention 26%, 11% in abortion, and 26% in RFM. In contrast to the current study findings, Holt *et al*., (2011) identified abortion as a clinical sign of brucellosis in 94.4 percent of Egyptians..

Several known high-risk behaviors were common among self-reported practices in the Liban zone of Somalia's pastoral community. These includes, consumption of unpasteurized milk and use of bare hands when dealing with animals and aborted materials. This study found that unfavorable (50 percent) exposure risk practices outnumber preventive practices. Our findings is similar to Edao *et al*., (2020) and Kenea and Megersa, (2021). Regarding attitude towards these behaviors 93% of participants consume raw milk which is one of sources of infection for humans. However, 100%

consume cooked meat which reduce the risk of getting infected with *Brucella*. This goes along with Lakew *et al*., (2019) who reported that 99.9 percent consume raw milk and 97.99 percent cook meat.

According to our findings, 73% of participants had no education, while nearly 27% had at least primary and above. This result agrees with Obonyo, Mark and Gufu, (2015) who reported 77% of the participants had no education. Despite the disparity in education, our study found that individuals with no education outperformed those with primary, secondary, or higher education. It is consistent with the findings of Njenga *et al*., (2020). Furthermore, our findings support Kenea and Megersa, (2021) findings that, when compared to the average knowledge score, participants with primary school had a higher knowledge score than their counterparts.

6. CONCLUSION AND RECOMMENDATIONS

The overall prevalence of brucellosis in the recent study was 3.7% in animals and 2.0% in livestock kepers in Liban zone of Somali region, Ethiopia. Despite the low level prevalence, there was a significant association between human seopositivity and exposure risks suchas disposal of fetal membrame or aborted materials, suggesting the zoonotic risk of thedisease at household level. Age and herd history of RFM, were found to be risk factors for brucellosis in animals. Gender, presence of fever or joint pain and disposal methods of aborted or RFM were also significantly associated with *Brucella* seropositivity in humans. The recent drought in Somali region triggered by climate change has caused interspecies mixture, migration of pastoralists, and congregation of pastoral communities at one place with other factors were likely contribute to the occurrence in the disease in the study area. Assessing the KAP of the herders showed that education level and districts were significant towards attitudes of brucellosis. Existence of close contact between human and animals in pastoral community and wide prevalence of brucellosis in livestock remarkably indicated the potential risk of public health in this study. Moreover, lowland pastoral areas constitute an important source of small ruminants for highlanders and international markets, so that brucellosis require due attention.

Therefore based on the above conclusive remarks, the following recommendations are forwarded:-

- Feasible control strategy of the disease in respect of pastoral community and the sociocultural status through one health approach
- Enhanced public health education on brucellosis transmission is vital towards the prevention and control of the disease in Somali region.
- Raising public awareness with regard to the traditional practices that create positive behavioral change towards risk reduction and prevention methods of *Brucella* infection.
- Further an indepth studies at regional and country level is required to identify epidemiological hotspots and the circulating *Brucella* species by isolation and characterization for better monitoring of the diseases in human-animal interface.

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8. ANNEXES

Annex 1: Laboratory Procedure

A. Rose Bengal Plate Test

Procedure: -

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

1.30 µl serum was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.

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

3. Rock plate by hand for about 4 minutes

4. Examine for agglutination in a good light

5. Use magnifying glass when micro agglutination suspected

Interpretation

0 = no agglutination

+ = barely perceptible

++ = fine agglutination, some clearing

+++ = coarse clumping, definite clearing

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

B. ELISA Test

Preparation of Reagents

1. Diluting buffer

- The diluting buffer was prepared by adding 5 tablets of phosphate buffered saline (PBS), 0.5 ml phenol red indicator and 250µl of tween 20 to 500ml distilled water. The pH was in the range of 7.2-The buffer was stored at 4ºC until used in the next day.

2. Wash solution

- The wash solution was prepared by adding the contents of the ampoule of Na2HPO 4 and 1ml of Tween 20 to itres of distilled water then stored at room temperature until used in the next day.

3. Substrate buffer

Substrate buffer was prepared by

- Dissolving 1tablet of the substrate in 120ml of distilled water.
- The pH was within 3.9-4.4.
- These were prepared by the reconstitution of the positive and negative control samples included in the kit each with 1ml sterile distilled water and allowed to stand until an even suspension was obtained then stored at 4ºC until used in the next day.

4. Chromogenic

- Chromogenic was prepared by dissolving 2 ABTS tablets in 1ml sterile distilled water and stored in the dark at 4ºC until used in the next day.

5. Stop solution

Stop solution was prepared by

- Diluting the contents of the ampoule of sodium azide with 500ml of distilled water
- Then stored at room temperature until used in the next day.

The procedure

i. A primary dilution of 1/40 of all test and control sera was made by the addition of 25µl serum to 1ml of diluting buffer.
ii. The plate was prepared by addition of 190µl of the diluting buffer to all wells.
iii. A 10µl of each of the primary diluted samples was added to all prepared wells.
iv. This gave a final dilution of 1/200. Columns 11 and 12 were left for the serum controls.
v. A 20µl of the primary diluted positive control was added to each of the wells in column 11, and 20µl of the primary diluted negative control was added to each of the wells in column 12 except well H12 which was left without sample so as to blank the plate.
vi. The plate was then covered with the lid and incubated at 37ºC for 1 hour.
vii. The content was then shaken out and the plate was rinsed 5 times with the washing solution and then thoroughly dried by tapping the plate on absorbent paper towel.
viii. The conjugate solution was then prepared by adding the content of the ampoule to 11ml of the previously prepared diluting buffer.

ix. A 100µl of the conjugate solution was added to all wells. The plate was then covered with the lid and incubated at 37ºC for 1 hour.

x. The content was then shaken out and the plate was rinsed 5 times with the washing solution and then thoroughly dried by tapping the plate on absorbent paper towel.

xi. The substrate solution was prepared immediately before use by addition of 300µl of ABTS (2, 2'-Azinobis (3-ethyl benzo thiazoline-6-sulfonic acid) diammonium salt) chromogen to 12ml of substrate buffer plus 60µl of the substrate (hydrogen peroxide).

xii. Mixed well and a 100µl of it was added to all wells. The plate was then left at room temperature for 12 minutes. A 100µl of the stopping solution was then added to all wells.

xiii. The plate was then read in the microtire plate reader at 405nm blanked on well H12. A positive/negative cut-off was calculated as 10% of the mean of the optical density (OD) of the 8 positive control wells. Any test sample gave an OD equal to or above this value was considered as being positive.

For camel samples

- Test effectiveness: the average value of positive control ≥ 1.00; the average value of negative control ≤0.10.
- The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15
- Negative Result: if the OD value < CUT OFF, the sample is Camel *Brucella* negative.
- Positive Result: if the OD value ≥ CUT OFF, the sample is Camel *Brucella* positive.

For Small ruminants

Calculations:

- Mean PI of the positive control should be ≥ 80 %.
- Mean absorbance of the negative control should be ≤ 0.5.

Interpretation of results:

- PI= (1- OD sample / average OD negative control) ×100%

Samples:

- If PI ≤ 70%, the test samples are considered negative.
- If PI > 70%, the test samples are considered positive.

For Human

- Mean OD of the Positive Control should be ≥ 1.00.
- Mean OD of the Negative Control should be ≤ 0.20.
- CUT OFF value = Negative Control + 0.15

If the Positive Control value is ≥ 1.00, and the Negative Control value is ≤ 0.20, the test is valid, otherwise, the test is invalid.

- If OD of Samples < CUT OFF, the test samples are considered negative.
- If OD of Samples ≥ CUT OFF, the test samples are considered positive

Annex 2: Questionnaire

General Questionnaire

District _____Kebele _____ Date: _____
Agro-ecology_____ (midland, semi-arid, arid)
Respondent name _____ Sex: _____ Age _____
Education level _____ Family size_____

Livestock ownership

	Animal species					
	Cattle	Sheep	goats	camel	equine	dogs
Yes / No						
Number (optional)						

1. Have you observed the following clinical symptoms in your herd during the past one year?

	Clinical Symptoms				
	Abortion	Still birth	Retained placenta	Repeat breeding	Others
Yes / No					
Species affected					
Assumed causes					

2. Have you encountered illness in human with symptoms fever, joint pain, and fatigue and skin lesion in your family or village during the past one year? (Yes / No)

_____

Which diseases you know can show signs like fever, joint pain, fatigue?

_____

Would you mention diseases transmitted through milk or meat consumption to humans?

_____

KAP questions

Knowledge Questions

1. Have you ever heard about brucellosis?
   A. Yes B. No
2. If yes, from where do you heard?
   A. Health worker B. Veterinarian C. Media D. Patient E. Community F. Education
3. Is brucellosis an animal health problem in this area?
   A. Yes B. No
4. Which animal species affected by brucellosis?
   A. Sheep B. Goat C. Cattle D. Camels E. Equine F. Dogs
5. Do you know clinical signs in camels, sheep and goats?
   A. Abortion B. RFM C. Hygroma D. Sterility E. Orchitis
6. Does it transmit from animal to humans?
   A. Yes B. No
7. What are modes of transmission to animal?
   A. . Contact B. Mating C. Contaminated feed and water D. Licking discharge E. Others (Specify)
8. Does it transmit from animal to human?
   A. . Yes B. No
9. Which Mode of transmission to human?
   A. Contact of infected item B. Raw milk consumption C. Through blood and meat
10. How can you tell if someone affected by Brucellosis?
    A. Fever B. Joint pain C. Sweating D. Headache E. Fatigue F. Swelling of testicles
11. What is treatment means in animals?
    A. Drugs B. Herbs C. Religion

12. What is prevention means?

A. Vaccine B. Isolation C. Care during disposal of aborted tissues D. others (Specify)

Attitude Questions

1. Do you think boiling milk prevent brucellosis?

   A. Yes B. No C. Not Know

2. Do you think cooking meat prevent brucellosis?

   A. Yes B. No C. Not Know

3. Do you think washing hand after contact with animal/product is useful?

   A. Yes B. No C. Not Know

4. Do you think you need more information on brucellosis?

   A. Yes B. No C. Not Know

5. Do you think brucellosis in human is treatable?

   A. Yes B. No C. Not Know

6. Do you think brucellosis in animals is treatable?

   A. Yes B. No C. Not Know

7. Do you think drinking milk from aborted sheep and goat or camel has health problem?

   A. Yes B. No C. Not Know

8. Do you think consumption of raw meat (organ) has health problem?

   A. Yes B. No C. Not Know

9. Do you think disposing aborted material in the field has problem?

   A. Yes B. No C. Not Know

Practical Questions

1. Do you and your family drink raw milk?

   A. Often B. Sometimes C. Never

2. Do you drink milk from aborted Camels, Sheep and Goats?

   A. Often B. Sometimes C. Never

3. Do you and your family eat fresh liver, kidney, spleen, or tongue?

   A. Often B. Sometimes C. Never

4. Do you and your family eat raw meat?

   A. Often B. Sometimes C. Never

5. Do you and your family consume fresh urine?

A. Often B. Sometimes C. Never

6. Do you assist delivery (kidding/lambing) with your bare hand?
   A. Often B. Sometimes C. Never
7. Do you remove retained placenta with bare hand?
   A. Often B. Sometimes C. Never
8. Do you handle aborted fetus with bare hand?
   A. Often B. Sometimes C. Never
9. Do you slaughter animal in barn/backyard?
   A. Often B. Sometimes C. Never
10. Do you milk sheep and goat, camel?
    A. Often B. Sometimes C. Never
11. Do you slaughter sick animal in the farm?
    A. Often B. Sometimes C. Never
12. Do you boil milk for consumption?
    A. Often B. Sometimes C. Never
13. Do you separate sheep, goat, and camel during parturition?
    A. Often B. Sometimes C. Never
14. Do you isolate aborted or sick animals?
    A. Often B. Sometimes C. Never
15. Do you wash your hands with soap after assisting delivery?
    A. Often B. Sometimes C. Never

Annex 3: Consent Form

Voluntary Participation: You are free to join this is study or not to join. You may leave the study at any time, for any reason. If you decide not to join or to drop out, you will not lose any health care services you are entitled to a hospital. You will not get any direct benefit or payment for being in this study, but you will help us known more about this disease.

Procedure and Confidentiality: if you or child chooses to be in this study, we will draw 4 ml of the blood from the vein in your, his or her arm. This blood will be tested for disease called Brucellosis, which is caused by bacteria at Regional Veterinary Laboratory in Jigjiga. Test may show us you or your child may have been sick with Brucellosis before or is sick with it now. We will also ask you and your child question related to knowledge, attitude and Knowledge of

Brucellosis. Neither of you have to answer the questions if you don't want to. Only researchers involved in this study will be allowed to work your blood and see your information. Your name and anything that can identify you will be taken off the test results and the questions you were asked before it is looked at and reported.

Risks: Except minor pain, bruising and bleeding that may be part of taking blood, there are minimal risks for being in this study. In rare cases, an infection can results from drawing blood

Benefit: You will not receive any benefit from this study, information obtained from this study may help the Ministry of Health decide when and where Brucellosis disease may occur. In addition, the result will be provided to the districts as soon as possible to find some medications.

Consent: This is study is had been explained to me. I have had a chance to ask questions. I have been informed that it is my free choice to be in this study and if I join the study, I can drop out anytime without any penalties.
If you agree to participate in this study please sign, print/thump print here.

_____

Name of the participants_____

Date _____

Annex V: Photos

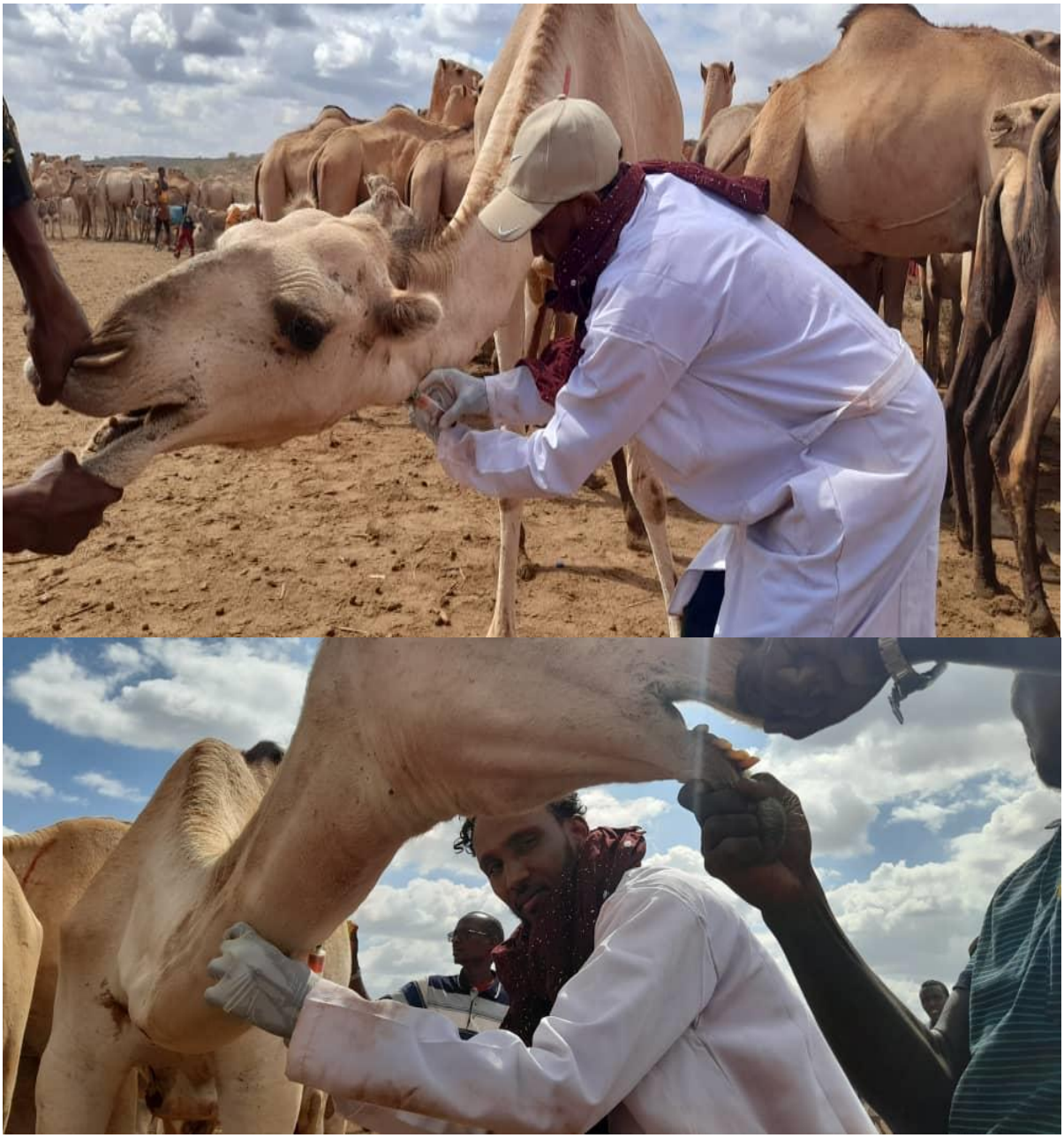

Fig1: Photo illustrating sample collection from camel

Fig 2: Harvesting and labelling of Serum at health post of the study areas at night.

Fig3: Assessing animal owners about KAP at Helaweyn Kebele of Dolo Ado district.

Fig4: Demonstration of RBPT at field health post of the study area (Dolo Ado)

Fig 5: ELISA demonstration at Jigjiga Regional Diagnostic and Research Laboratory (JRDRL)

Fig6: Different reagents used during demonstration of ELISA procedure.

Fig 7: ELISA at JRDRL