



**JIGJIGA UNIVERSITY
SCHOOL OF GRADUATE STUDIES**

**SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF SMALL
RUMINANTS BRUCELLOSIS IN SELECTED DISTRICTS OF SAHIL REGION,
SOMALILAND**

**A THESIS PROPOSAL SUBMITTED TO THE DEPARTMENT OF ONE HEALTH
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Abstract

Brucellosis is an imperative zoonotic disease that causes vast economic losses to the livestock owners and is of major public health concern worldwide. It is a chronic infectious disease of livestock, rodents, marine animals and human beings. Brucellosis upsets both public and animal health as well as production, and is prevalent in many regions of the world. The disease is caused by non-motile, facultative intracellular Cocco-bacilli of genus *Brucella*. The two specific isolates of *Brucella*, *Brucella melitensis* and *Brucella ovis*, cause brucellosis in small ruminants. *Brucella ovis* causes the disease in sheep while *B. melitensis* is the etiologic agent of brucellosis in man, sheep and goats. Direct contact with infected animal secretions, inhalation of the organism, ingestion of contaminated food, and poor hygienic practices favor the transmission of brucellosis between animals and humans. Brucellosis affects the reproductive tract of animals which is demonstrated by late term abortions, retention of placenta in the case of female animals, epididymitis and orchitis in males. The disease is also characterized by infertility and reduced milk production. The diagnosis of brucellosis focuses on culture, serological tests and molecular investigations. Because of the high relapse rate associated with the disease, the use of a multidrug therapy is recommended. Brucellosis can be prevented by implementing appropriate animal-disease-control measures; avoiding the consumption of undercooked meat and unpasteurized dairy products; and using appropriate barrier precautions to exclude exposure to aerosols in humans.

Keywords: brucellosis, *brucella melitensis*, *brucella ovis*, small ruminants, zoonosis, humanans.

1 INTRODUCTION

Brucellosis, caused by bacteria from the genus *Brucella*, is an infectious disease known to induce infertility and abortions in various animal species. (Khan, Zahoor, & disease, 2018; Khurana et al., 2021). According to numerous researchers, this ranks among the most significant zoonotic diseases globally. (Franc, Krecek, Häsler, & Arenas-Gamboa, 2018; Godfroid, 2017). Besides Somaliland, the disease is prevalent across Africa, Asia, and numerous Mediterranean countries. It is primarily caused by *Brucella melitensis* in small ruminants, with *Brucella ovis* also playing a significant role in rams, ewes, and goats, particularly in orchitis and epididymitis. (Carlos et al., 2022; Saxena et al., 2018). *Brucella suis* and *Brucella abortus* have been sporadically reported in small ruminants. Brucellosis typically spreads through contaminated food or drink, sexual intercourse, or direct contact with infected uterine or placental secretions. (Berhanu, Pal, & Protection, 2020). Various animal and environmental factors, including species, geographical location, and husbandry practices, influence the transmission of the disease. (Coelho, Díez, & Coelho, 2015).

Due to its effects on abortion, infertility, decreased milk output, the requirement to cull infected animals, and the costs associated with treatment, brucellosis is a significant contributor to financial losses (Pal et al., 2017). Brucellosis significantly affects individuals due to the loss of employment and money it causes. However, it is crucial to remember that approximately the 5 million individuals reside in Somaliland, and the rearing and trading of livestock is a substantial income source for numerous individuals (Wanyoike et al., 2023). Seventy per cent of the population finds work in the livestock business, accounting for thirty per cent of the GDP and eighty-five per cent of the country's export earnings. With livestock losing an average of US \$3.4 billion annually, Somaliland has a high prevalence of brucellosis. Similarly, in India, losses among humans were estimated to be \$6.39 million and \$2.67 million, respectively (Shome et al., 2020). There are various ways to control brucellosis, such as keeping an eye out for hotspots, reducing risk factors, quarantining infected animals, and making vaccinations necessary (Lado et al., 2016; Rahman et al., 2020).

A significant portion of the population in Somalia consumes raw milk and uncooked meat (Adebayo et al., 2017). The existence of small ruminants infected with *Brucella* positions a potential health hazard to consumers in the nation. Currently, Somaliland still

needs a thorough plan to control brucellosis. The risk of the disease spreading among animals is increased by the absence of a vaccination program, which might cause significant financial losses for the livestock industry and have a negative effect on public health. There is a need to identify risk factors to establish measures to avoid disease transmission in small ruminants raised in organized farming systems. This is especially significant because the government does not have a vaccination strategy for these animals. Hence, this research will aim to estimate the prevalence of small ruminant brucellosis and assess the associated risk factors of the disease in sheep and goats and in livestock systems in general.

1.1 Research Problem Statement

The Sahil region in Somaliland is facing a significant threat from brucellosis, a disease that not only endangers livestock but also poses risks to public health. Although there is anecdotal evidence suggesting widespread prevalence, there is a lack of studies investigating the seroprevalence of the disease and its associated risk factors in small ruminants. This information gap hinders effective disease prevention and control efforts, leading to increased economic losses and public health risks. Sheik - Mohamed, Velema, and health (1999) found substantial seroprevalence in neighbouring small ruminants, suggesting similar trends in Sahil. This shows that brucellosis is endemic in the Sahil region. Middlebrook et al. (2022) state that environmental factors such as high temperatures and dry conditions may exacerbate *Brucella* spp. transmission in livestock. Virhia (2020) discovered that socioeconomic factors such as nomadic pastoralist livelihoods and informal livestock trading networks may accelerate the spread of brucellosis within and across animal populations and pose human health risks. According to studies by Maree et al. (2014), the Sahil region lacks diagnostic equipment, immunizations, and skilled veterinary personnel. Franc et al. (2018) state that a livestock production system based on traditional husbandry and inadequate biosecurity measures may exacerbate brucellosis transmission in small ruminant populations. Given these evidence gaps and the possible impacts of brucellosis on livestock production and public health, examining the disease's seroprevalence and risk factors in Somaliland's Sahil region is necessary. These gaps must be addressed to minimize brucellosis, economic losses, and public health prevalence.

1.2 Objectives of the Study

General objective

To estimate the seroprevalence and associated risk factors of small ruminants' brucellosis in selected Sahil Region, Somaliland districts.

Specific Objectives

- To estimate the seroprevalence of small ruminant brucellosis in the study area.
- To assess the potential risk factors that are associated with the occurrence of brucellosis in small ruminants in the study area

1.2 Significance of the Study

Brucellosis poses a significant threat to both animal and public health in Somaliland, especially in the Sahil region where small ruminants are essential for many households. (Edao, 2021). Understanding the seroprevalence and risk factors of brucellosis in this area is crucial. This study on small ruminants will clarify brucellosis epidemiology and aid in developing effective control methods. Evaluating its distribution and risk factors will assist policymakers and veterinarians in managing the disease, which imposes substantial economic losses on livestock farmers in Sahil (Mohamud et al., 2020). Control and immunization efforts will be analyzed to optimize resource allocation and interventions aimed at reducing losses from culling infected animals, abortion, infertility, and reduced milk production (Ali et al., 2019). Given its zoonotic potential, understanding how brucellosis spreads from animals to humans is crucial for public health initiatives to prevent human cases. This study aims to improve the health and well-being of both humans and animals in Somaliland, benefiting the entire country.

2 LITERATURE REVIEW

2.1 Small ruminants Brucellosis

Only North Europe, North America, Southeast Asia, and Oceania are immune to brucellosis, which has a worldwide spread (Wernery, 2014). The disease is endemic in nearly all countries in the North America (WANA) region and West Asia, including Sudan, Somaliland, Algeria, Syria, United Arab Emirates, and Iran, as well as Latin American countries, including Mexico, Peru, Argentina, and Brazil, where it is often reported. Some South Asian countries, like Bangladesh, Pakistan, and India, have endemic diseases. Substantial economic losses, particularly in cattle, are caused by the disease, a significant public health concern (Narladkar, 2018).

The first reports of brucellosis came from the Mediterranean region, specifically Malta Island, and its historical relationship with military engagements was established. Britain had a military presence on this island during the 18th and 19th Centuries (Biselli et al., 2022). During this time, several British doctors meticulously recorded cases of sickness among the stationed soldiers. The responsibility of examining these incidents fell on physician David Bruce. He identified the organism as *Micrococcus melitensis* after successfully isolating it from four fatal cases in 1887 (Dossey, 2010). After identifying specific *Brucella melitensis* antibodies in humans and animals, Wright and Smith explained that brucellosis was a zoonotic disease in 1897. This was followed by the isolation of *B.* by Themistocles Zammit (1864–1935). goat's milk containing *melitensis* (TASEW, Motbynor, & Tsegaye, 2023).

Borrelia brucellosis in cattle, caused by *B. peritonitis*, is an endemic disease in animals worldwide. The most widely spread type of abortion in animals is still the fetus. Four species of bacteria are the primary cause of human brucellosis. *A. melitensis*, *B. pregnancy termination*, *B. I. suis*, and *B. the canis*. One of these is *B.* Up to 90% of all brucellosis cases with *B. melitensis* are still caused by man globally. Type 1 *melitensis* is most common in India and Spain, type 2 in northwest Greece, and type 3 in Turkey (Corbel, 2020). This is the infectious *B.* 10 organisms are *melitensis* low (Boral et al., 2008). Infection leakage in industrialized countries has been reported, making brucellosis in wild animals a more significant problem (Sharma & Ganguly, 2017).

The majority of cases of brucellosis in humans occur as a result of eating raw or undercooked traditional delicacies such as liver (Berhanu et al., 2020) or spleen or other products made from unpasteurized milk (Owusu-Apenten & Vieira, 2022). According to Owusu-Apenten and Vieira (2022), camel milk is considered the primary cause of infection in countries in the Middle East. Tissue transplantation and sexual contact are two very unusual routes of transmission from humans to humans (Kiros, Asgedom, Abdi, & Sciences, 2016). Additionally, the disease can be transmitted by inhalation of the organism, damaged skin and mucous membranes of the conjunctiva, as well as through contact with infected animals' urine, excrement, blood, and vaginal discharge. Farmers, veterinarians, para-veterinarians, dairy workers, shepherds, and laboratory staff are all at risk of contracting brucellosis while on the job. One of the most prevalent illnesses that may be acquired in a laboratory setting is brucellosis, as shown in studies. Brucellosis is becoming a prevalent imported disease in the industrialized world due to the rise in worldwide travel (Addis, 2015).

Brucellosis is a common, occasionally disregarded ailment in Somaliland. As a result, *B. Melitensis* is widely prevalent in Somaliland and is a major cause of abortion in small ruminants. This harms the country's overall animal protein supply and poses a significant risk to human health (Ahmed, 2021). Sheep brucellosis may be classified into two primary forms: classical brucellosis and ram epididymitis. The former is attributed to the non-zoonotic pathogen *B.ovis*, whereas goat brucellosis poses an equivalent threat to public health. According to Seleem, Boyle, and Sriranganathan (2010), brucellosis is the most common zoonotic disease, with around 500,000 new cases reported in humans worldwide each year.

Carlos A Rossetti, Arenas-Gamboa, and Maurizio (2017) state that brucellosis is a significant global health issue, affecting several regions such as the Mediterranean, the Middle East, India, Latin America, Africa, Mexico, and Asia. Syria has the most significant yearly incidence of human cases, with a recorded rate of 1603 cases per million per year (Abdulrazzak et al., 2024). The prevalence of human infection is significantly higher in Peru, Kuwait, and Saudi Arabia compared to sub-Saharan Africa, primarily due to underreporting and inadequate surveillance (Pappas, Papadimitriou, Akritidis, Christou, & Tsianos, 2006). Only a few countries have entirely eradicated the disease, brucellosis being so widespread. The disease is still endemic in a large number of regions. One hundred forty-three countries worldwide have reported cases of bovine brucellosis (Berger & team, 2023). Bovine brucellosis has affected the majority of African countries. The disease has been shown to

have the highest prevalence in Asia in Iran, India, Pakistan, Bangladesh, Sri Lanka, Mongolia, and Hong Kong.

2.1.1 Etiology

Brucellae are Gram-negative, facultative, non-spore-forming, intracellular coccobacilli or rods; that lack capsules, flagellae, and endospores. Brucella organisms grow slowly, but can be enhanced by using enriched media, such as Ferrell's media supplemented with 5% horse serum and six added antibiotics (Edao, 2021). Brucella species differ in host specificity and virulence. *Brucella melitensis* (goats and sheep), *Brucella abortus* (cattle), *Brucella suis* (swine), and *Brucella canis* (dogs) are major species (Moreno, Cloeckaert, & Moriyón, 2002). These species are genetically close but have different host and pathogenicity characteristics. Brucella species, tiny, Gram-negative, facultative intracellular bacteria, may survive and proliferate in host immune system phagocytic cells. Brucella's outer membrane contains LPS and OMPs, which are essential for virulence and host interactions (Gopalakrishnan et al., 2016). Brucella's intracellular lifestyle allows for evasion of host immune defences and the creation of persistent infections, which contributes to the disease's persistence. Brucella species have virulence elements that aid host colonisation, invasion, and immune evasion. The type IV secretion system (T4SS) translocates bacterial effectors into host cells, increasing intracellular survival and reproduction (Ke, Wang, Li, Chen, & microbiology, 2015). Brucella lipopolysaccharides (LPS) decrease pro-inflammatory cytokine production and promote immune evasion (Gopalakrishnan et al., 2016). Brucella species display genetic variety, with numerous genotypes and lineages in host populations and locales. Multi-locus variable number tandem repeat analysis (MLVA) and whole-genome sequencing (WGS) have helped characterise Brucella strains and study their epidemiology (Pelerito et al., 2021).

2.1.2 Epidemiology

In 1905, Zammit, who also confirmed the zoonotic origin of *B. brucellosis*, documented the initial occurrences of brucellosis outbreaks in goats on the Mediterranean island of Malta. Hussain *et al.* (2020) successfully isolated *melitensis* from goat milk in Pakistan. They discovered that 3.0%, 1.6%, and 0.95% of serum samples from humans, sheep, and goats tested positive for brucellosis.

Hosein (2015) comprehensively evaluated serological tests used to identify antibodies to *B.*

in goats. The presence of melitensis was determined by examining 2213 goat sera samples, with 716 (32.35%) of these samples proving positive using the PAT and CFT methods. Ebid, El Mola, and Salib (2020) conducted a study in Jordan, randomly testing 1100 goats from 69 herds using RBPT and CFT. They determined that I-ELISA had the highest sensitivity (96.2%) and specificity (99.7%). 305 (or 27.7%) goats tested positive for Brucella antibodies out of 1100. In Brazil, on testing 400 goats in the Bahia region by RBPT and STAT, Etsebeth (2017) found just 3 (0.75 percent) positive tests, whereas in West Cameroon, on testing 804 bovine serum samples, Shey-Lysholm *et al.* (2022) found a prevalence of 1.64 per goat and 4.88 per goat, respectively.

Benkirane (2006) in an investigation of brucellosis incidence and prevalence in the WHO Western Asia and North Africa region (WANA) revealed a significant increase in abortions in Gherdaia in 1984. As a result, between 1986 and 1989, the seropositivity rate in sheep was roughly 2.2% per flock, while in goats, it was 12% per flock. The prevalence of the disease in sheep herds was 43.5%, and in goat herds it was 42%. Berri, Rekiki, Boumedine, and Rodolakis (2009) conducted a study where they analyzed 167 blood samples to detect the presence of *B. melitensis* and *Chlamydiophila abortus* in aborting sheep from Turkey. Of the 167 samples, 71 cases (40.11%) tested positive for *B. melitensis* and *Chlamydiophila abortus*.

M. Rahman *et al.* (2011) examined the occurrence of brucellosis in ruminant animals, including buffaloes, cattle, sheep, and goats, across five distinct regions. They discovered that 9 (5.38%) of the total cases tested positive for *Chlamydiophila abortus*. Mymensingh, Sirajgonj, Bagerhat, Bogra, and Gaibandha are cities located in Bangladesh. A total of 550 serum samples from 105 buffaloes, 188 cattle, 127 goats, and 130 sheep were confirmed using RBT and I-ELISA. A thorough questionnaire was employed to collect epidemiological data on the animals. The final serological prevalence estimates for the samples were as follows: 2.87% for buffaloes, 2.66% for cattle, 3.15% for goats, and 2.31% for sheep. Chaulagain and Bowen (2016) conducted a study in the Kailali area of Nepal where they examined 116 blood samples using the plate agglutination test. They discovered that the prevalence of the disease was considerably greater in females compared to males in cattle, goats, and sheep. The recorded seroprevalence rate was 2.6%.

Obonyo (2018) studied the Tras-os-Montes-e-Altodouro region of Northeastern

Portugal. They studied 278,097 small ruminants and 5,466 farmers using RBPT (Rose et al. Test) and CFT (Complement Fixation Test). Four hundred eighty-seven farmers, accounting for 8.9% of the sample, had at least one serologically positive animal. The prevalence of positive animals ranged from 8.2% to 9.7%. Sharifi et al. (2014) conducted a study in Kerman province, southeast Iran, to investigate the seroprevalence of brucellosis in small ruminants and identify flock-level factors associated with seropositivity. A total of 1767 sheep and 1233 goats, all older than 18 months, had their serum samples collected randomly in October and November 2011 from a pool of 300 flocks. The sera were initially examined for the existence of anti-Brucella antibodies using the Rose-Bengal test. The positive sera were further assessed using the Wright and 2-mercaptoethanol Brucella agglutination tests.

Gitonga (2015) collected data on flock-level factors likely linked with the seroprevalence of brucellosis within the flock were collected using a questionnaire. Using multivariable logistic models, we checked if the relationships were statistically significant. At least one animal in sixty-three flocks tested positive for the virus (21.00%; 95% CI). Serum prevalence within flocks averaged 3.10 percent (95% CI). The prevalence of brucellosis in sheep and goats in the Northern, Ashanti, and Greater Accra regions of Ghana was studied by (Jarikre et al., 2015). Tissues/swabs (319) and serum samples (370) were collected from sheep and goats between 1 and 4 years of age. These were screened for brucellosis using the Rose Bengal Plate Test (RBPT) and the Modified Ziehl-Neelsen (MZN) staining procedure. While 17.0% were positive with modified ZN staining, a seroprevalence of 13.3% was recorded. There was a slightly higher seroprevalence of over two years in goats (10%) and female animals (7.0%), but the highest prevalence was in WAD breeds (63.1%) and female WADs (2.7%). We found more seropositive animals in the Ashanti region than in other regions.

Some export abattoirs for sheep and goat products have their brucellosis seroprevalence studied by (Kolo et al., 2019). Using a rigorous random sample approach, 450 sera were collected from goats at those chosen export abattoirs. 1.56% (N=7) of the samples tested positive for the Rose Bengal Plate Test, employed as a screening test. Out of the total samples tested, only 1.11 percent (N=5) showed positive results after further confirmation testing utilizing CFT.

Tegegn, Feleke, Adugna, and Melaku (2016) conducted a study investigating the

seroprevalence of small ruminant brucellosis and its correlation with notable reproductive health issues. In addition to a questionnaire study, we collected serum samples from three randomly selected peasant groups. The samples included 272 from goats and 142 from sheep. The seroprevalence in sheep and goats was 13.7% in total, with goats having a substantially higher prevalence of 15.4% compared to sheep, which had a prevalence of 10.6%. The screening test was the Modified Rose Bengal Plate Test (RBT), while the reactors were verified using the Complement Fixation Test (CFT).

2.1.3 Occurrence

Brucellosis is a global health hazard with a variable worldwide occurrence pattern. Different species of *Brucella* bacteria cause this sickness in small ruminants like sheep and goats, compromising animal welfare and public health (Edao, 2021). Many developing nations, especially in Africa, still have brucellosis due to various factors. Brucellosis occurs in animal herds due to insufficient disease management resources, poor veterinary infrastructure, and traditional husbandry techniques that spread illness (Franc et al., 2018). Small ruminants in Ethiopia, Kenya, Nigeria, and Somalia have high brucellosis seroprevalence rates. The prevalence of brucellosis in African cattle necessitates extensive management (M. Ducrototy et al., 2017). Brucellosis prevalence is influenced by climate, husbandry, and socioeconomics. Due to communal grazing, inadequate vaccination regimens, and limited veterinary services, small ruminant populations are endemic to Brucellosis (Cruz, 2018). Since people can get the infection through infected animals or contaminated animal products, the occurrence of brucellosis in small ruminants has implications for public health. Unpasteurized dairy and occupational exposure are linked to human brucellosis in Somaliland's Sahil region, where animals are endemic (Addis, 2015).

2.1.4 Sources of Infection and Transmission

Direct contact with infected animals, consumption of contaminated animal products, and exposure to infected environments are the main modes of Brucellosis transmission. To stop animal populations and people from contracting the disease, control measures must understand infection sources and transmission mechanisms (Coelho et al., 2015). Direct contact with infected animals is brucellosis transmission. Infected animals shed bacteria in placental tissues, vaginal discharges, and milk, making intimate contact with individuals infected a high risk factor. Contact with contaminated materials during parturition, such as aborted foetuses and placental membranes, increases the risk of transmission to susceptible

animals and humans (Gutema Wegi, 2020).

Drinking unpasteurized milk and cheese from infected animals can spread brucellosis. The high Brucella germ poses a public health risk counts in raw milk, particularly during acute infection (Ndaki, 2022). Consuming unpasteurized dairy products in cattle-endemic areas endangers human health and perpetuates the disease cycle. Brucella organisms may live in the environment for long periods, causing direct human and animal transmission. Bacteria are stored in pastures, water sources, and fomites, allowing host transmission (PLAN, 2005). Livestock grazing in contaminated areas or contact with contaminated instruments and facilities increases the risk of developing brucellosis via environmental exposure. Vector-borne brucellosis transmission. The major modes of transmission are direct contact and consumption of contaminated things. Brucella transmission between animals is facilitated by some arthropod vectors, such as ticks (Ma et al., 2024). Vector-borne transmission is rarer than direct contact and ingestion, although it can spread disease in certain ecosystems. Zoonotic Brucellosis is a disease that may transfer from animals to humans. The risk of zoonotic transmission is increased by occupational exposure, particularly among individuals working close to infected animals, such as farmers, veterinarians, and abattoir workers (Klous et al., 2016). Consumption of contaminated animal products causes most human infections.

2.1.5 Risk Factors in brucellosis

2.1.5.1 Agent factor

The intracellular pathogen Brucella may live and multiply inside phagocytic cells. Additionally, depending on factors including the month, temperature, and sunlight exposure, it can stay on fetal tissues and in the soil or plants for a variable length, ranging from 21 to 81 days (Xavier et al., 2010). Its ability to endure the phagolysosome's hostile environment contributes to its survival and reproduction capabilities. The unusual non-endotoxin lipopolysaccharide (LPS) that Brucella has serves to modulate the host immune response and affords resistance to antimicrobial assaults. Brucella relies on this specific lipopolysaccharide to survive and reproduce within the host, making it a potent virulence factor (HASAN, KADHUM, & ALASEDI, 2021).

2.1.5.2 Host and reservoir factors

Numerous domestic and wild animals, even humans, are susceptible to small ruminant

brucellosis. Age, breed, sex, immunological state, and pregnancy status are a few of the host factors affecting an animal's vulnerability to Brucellosis (Coelho et al., 2015). Age is considered one of the most significant risk factors for brucellosis among these factors. According to Suryawanshi et al. (2014) and Melese et al. (2016), the risk of infection is higher in fully-grown animals. This is due to the higher presence of sex hormones and carbon 4-erythritol sugar in adult animals, which promotes *Brucella* organism growth and multiplication (TASEW et al., 2023).

For example, sexually mature animals prefer male and female reproductive organs when infested with *Brucella* (TASEW et al., 2023). *Brucella* primarily targets the mammary glands, placenta, and epididymis in sexually mature animals. Pregnant females are more susceptible to infection among sexually mature animals, and this vulnerability rises as gestation age advances (Hellemans et al., 2008). Allantoic fluid, which provides an optimal environment for the growth of *Brucella*, has influenced the tendency of the bacteria to localize in the reproductive tract of pregnant animals (Akeberegn & Dawit, 2018). When comparing species, the prevalence of Brucellosis is higher in goats than in ten sheep. This might be attributed to goats having a longer duration of excretion of the organism than sheep.

Some of the most essential mechanical vectors in the transmission of brucellosis are carrier animals, which include wolves, foxes, cats, and dogs. By bringing contaminated materials such as retained fetal membranes and aborted fetuses, they contribute to the disease's spread and lead to environmental pollution. In addition to facilitating the pathogen's survival and persistence in the environment, the organisms carried by these animals can reproduce inside themselves and then release the germs into the surrounding environment. Therefore, the risk of infecting vulnerable animals significantly increases (Pavlik et al., 2009).

2.1.5.3 Farming Practices and Environmental Influences

Brucellosis in small ruminant animals is mainly caused by inadequate farming methods, poor farm cleanliness, increased animal movement and contact, and sharing grazing pastures (Mutua, 2017). The disease's prevalence and the challenges of controlling infection within the population are significantly influenced by the size of the flock and the density of animals. Increased flock size and greater animal concentration can result in heightened contact among flock members, particularly when grazing grounds are shared or where

cleanliness and infection control measures are inadequate (Health & Journal, 2014).

According to Gul and Khan (2007), there is a significant risk of brucellosis transmission between various species when goats, sheep, and cattle are kept together. A positive correlation exists between brucellosis and grazing practices that allow animals to roam freely and bring in animals from outside, particularly those with uncertain health conditions (Ng'ang'a, 2022). Extensive management also increases the likelihood that the disease may manifest in animals. This might be due to the unrestricted contact between animals in large-scale management systems, which could aid in the spread of brucellosis.

Adem and Duguma (2020) found that in filthy environments when aborted foetuses are left open and accessible to livestock, predatory animals, and humans, the survival of the *Brucella* organism significantly impacts the epidemiology of the disease. The presence of foreign organic matter makes it such that *Brucella* bacteria may survive drying out, and they can even be found in soil and dust. However, regular sterilizing doses of gamma rays can quickly kill them, especially when total exposure is guaranteed, because they are pretty sensitive to ionizing radiation.

2.1.6 Pathogenesis

Small ruminants suffer chronic Brucellosis because to a complex host immune response and *Brucella* species pathogenesis. After entering the host, *Brucella* species form intracellular replication niches in macrophages and dendritic cells (Carlos Alberto Rossetti et al., 2022). *Brucella* spp. enter and survive in host cells using LPS, Opps, and the Type IV secretion system (T4SS). *Brucella* smooth LPS decreased host innate immune responses and increased bacterial survival (Huy, Nguyen, Kim, Reyes, & Kim, 2022). During infection, *Brucella* blocks phagosome lysosome fusion and alters host cell signalling pathways to escape immune surveillance (de Figueiredo, Ficht, Rice-Ficht, Rossetti, & Adams, 2015). Because of its intracellular lifestyle, *Brucella* may live in host tissues and produce chronic granulomatous lesions, particularly in the reproductive organs, lymph nodes, and mammary glands of small ruminants (Coelho et al., 2019). Small ruminants' brucellosis pathogenesis is marked by a lengthy subclinical phase and intermittent shedding of bacteria in bodily fluids such milk, vaginal secretions, and semen. Chronic carrier status can spread *Brucella* in animal populations, challenging disease control and eradication (Kaltungo et al., 2014). In a study by Gebretsadik and BISHOFTU (2016), *Brucella melitensis*, the leading cause of small ruminant

brucellosis, showed a high degree of tissue tropism, preferentially colonising the reproductive tract and mammary glands of small ruminants infected with the disease. Brucella isolates demonstrated genetic heterogeneity between circulating strains, highlighting the complexity of brucellosis pathogenesis in the area.

2.1.7 Clinical signs

Reproductive failure, abortion, infertility, mastitis, decreased milk supply, poor offspring delivery, and a high death rate in lambs and kids are the primary and prevalent clinical signs of brucellosis in female sheep and goats (Ali et al., 2019). According to Kaltungo et al. (2014), the retention of fetal membranes is a common complication following an abortion, which usually happens in the latter stages of pregnancy. In addition to inflamed joints and bursae, brucellosis can lead to orchitis and epididymitis in male animals.

Brucellosis infection has the potential to cause death in humans. Melitensis is a highly concerning infection, a pathogen known for causing severe infections and contributing significantly to global morbidity (Addis, 2015). As a systemic disease, it presents clinically in humans with a diverse array of symptoms, such as headaches, excessive sweating, chills, weakness, broad body pain, joint pain, organ failure, and signs of mental disturbance. Nevertheless, this is hardly observed in low-income countries (Mousa et al., 1988). The mortality rate is low, mainly when the patient receives adequate antibiotic treatment.

2.2 Diagnosis

2.2.1 Serological Tests and their comparative efficacy

The isolation of *Brucella* spp. is the most precise and definitive method for diagnosing brucellosis. The technique described by Padilla *et al.* (2010) is tedious and time-consuming, and it has low sensitivity when used for chronic infection, as Roy *et al.* (2016) noted. Implementing the practice of isolation in laboratories is similarly fraught with risks, incurs high costs, and poses significant challenges. Therefore, the diagnosis of brucellosis mostly depends on assessing the antibody response using serological tests. Utilizing a battery of tests is advised for serological diagnosis of brucellosis since each test has distinct benefits and drawbacks. Consequently, several experts evaluated the specificity and sensitivity of these serological tests.

The RBPT is a rapid agglutination test that detects the presence of IgG1, IgG2, IgM,

and IgA in a serum sample. RBPT is more specific than STAT, requiring only one serum sample dilution. This specificity is due to the acidic nature of the antigen, which reduces non-specific IgM agglutination responses and promotes agglutination by IgG1. This information is supported by studies conducted by (Barbuddhe, Vergis, & Rawool, 2020). Nevertheless, the test is vulnerable to both incorrect positive results (caused by antibodies that react with other substances) and incorrect negative results (caused by an excessive concentration of antibodies that interfere with the test) (Braunstein & gynaecology, 2002). The FAO/WHO Expert Council on Brucellosis in 1986 suggested using the RBPT as a preliminary test to screen for the disease. If the RBPT yields positive results, confirmation should be made using the CFT method, particularly in field settings. Brucellosis has been seen to exhibit excessive sensitivity, especially in animals that have been vaccinated and in instances of bacterial illnesses that cross-react, such as *Yersinia enterocolitica*.

Muñoz *et al.* (2005) conducted a study to determine the specificity of the Rose Bengal Test. The test had a specificity of 94.3% for individuals with no history of brucellosis, 91.7% for individuals exposed repeatedly to *Brucella* serum, and 76.9% for individuals infected with *Brucella* who had received appropriate treatment within 12 months. The Rose Bengal plate test and complement fixation test (CFT) were used to analyse serum samples from patients with brucellosis. The overall sensitivity of the test was found to be 92.9%. In a serological study by Refai (2002), sheep slaughtered during the Hajj season were examined. The study found that the RBPT test was more specific than the ELISA test since the ELISA test yielded a higher number of favorable results compared to the RBPT test. The RBPT demonstrated a sensitivity of 78.3% and a specificity of 81%, using the CFT as the reference standard. STAT is the most widely used test in the world for the diagnosis of brucellosis in men and animals. Sadhu *et al.* (2015) developed the test as a delayed agglutination diagnosis for low in humans. the test can generate false positive findings because it does not eliminate cross-reacting non-*Brucella*IgM antibodies that are created in response to immunological activation by respiratory and gastrointestinal bacteria. The test's specificity has been questioned due to a high rate of non-specific false positive reactions, and its use for international trade is discouraged (M. *et al.*, & immunopathology, 2016). It was also the primary test used for *Brucella* eradication programs in Norway, Denmark, and Sudan because it identified most infected non-vaccinated animals. Dongo (2015) compared the RBPT, STAT, and MRT to diagnose caprine brucellosis and found that the STAT offered a better serological diagnostic

tool for the disease. In a study comparing the RBPT, STAT, and Counter Immuno-Electrophoresis (CIEP), Hope, Kluver, Jones, and Condron (2000) examined 647 sheep sera samples using these tests. According to the study, STAT has a higher age effectiveness than other tests for identifying early illnesses. ELISA, the initial assay developed by Clark, Lister, and Bar-Joseph (1986), has been extensively used in veterinary medicine to diagnose various infectious diseases. The ELISA technique has commonly been employed for diagnosing various infectious conditions, such as brucellosis (Araj, 1999). The test possesses inherent advantages of having a high level of sensitivity and specificity, as well as being straightforward to carry out.

In 2015, Jimenez de Dieste-Perez, Blasco, De Miguel, Moriyon, and Munoz (2015) suggested using ELISA with crude S-LPS as a reliable diagnostic test for B. The serological test has been extensively employed for diagnosing brucellosis in animals and humans—Sheep infected with melitensis. It had a slower response to protein than S-LPS. In their study, Bányász, Antal, Dénes, and Disease (2023) found a range of specificity (95-99 percent) observed between S-LPS and a recombinant protein-based iELISA in many animal species. The sensitivity of Melitensis infection in sheep was determined to be 94.7%, indicating a high rate of accurately identifying positive cases. However, the specificity was 90.4%, indicating a lower rate of accurately identifying negative cases.

In their study, Saxena et al. (2018) compared ELISA and RBPT, STAT, CFT, and SDTH tests on animals with confirmed infections. The results showed that RBPT, STAT, CFT, and SDTH tests had similar diagnostic efficacies, with a 75-80% detection rate for infected animals. The I-ELISA and RBPT tests were determined to be the most sensitive methods for diagnosing brucellosis using sera from Brucella-free animals. ELISA identified 97-100% of infected animals and 99.8% of animals that tested negative in culture—infected with melitensis and (B). In a study conducted by the performance of three different tests, namely FPA, I-ELISA, and C-ELISA, was compared using 454 sheep serum samples and 251 goat serum samples. The results showed that the relative sensitivities of the three tests were 85%, 93.5%, and 88.5%, respectively. Similarly, the relative specificities were 96%, 94.5%, and 98.5%, respectively.

2.3 Treatments

The intracellular causal agents, limited treatment options, and possibility for zoonotic transmission make treating brucellosis in small ruminants problematic. While antimicrobial agents have been proven effective against *Brucella* species *in vitro*, their effectiveness *in vivo* is typically limited by low tissue penetration and persistent bacteria reservoirs (López-Santiago et al., 2019). Tetracyclines and rifampin are the most often used antibiotic therapy agents for acute brucellosis in small ruminants. Oxytetracycline and doxycycline reduce protein synthesis in *Brucella*-infected host cells, rendering them bacteriostatic (Kosgei, 2016).

Rifampin stops *Brucella* reproduction by inhibiting bacterial RNA synthesis. Tetracyclines plus rifampin therapy enhances treatment effectiveness and lowers brucellosis recurrence in brucellosis-infected animals. Chronic or persistent brucellosis may require ongoing antibiotic therapy to remove bacteria and prevent disease recurrence. This highlights the need for judicious antimicrobial use in veterinary medicine due to antibiotic-resistant bacteria (Mode, 2019).

In the treatment of brucellosis-infected small ruminants, supportive care and antimicrobial therapy are crucial. Nutritional supplements, appropriate housing, and biosecurity measures minimise stress and boost immune function, helping herds recuperate and reduce disease risk transmission (Gutema Wogi, 2020). *Brucella* eradication from chronically infected individuals, particularly in animals, is difficult despite treatment. Some regions use test-and-slaughter and vaccination efforts to control brucellosis transmission and disease prevalence in small ruminant populations. The effectiveness of these control measures depends on vaccination efficacy, coverage, and regulatory compliance (Refai, 2002).

Yusuf *et al.* (2017) found that while antibiotic therapy could temporarily suppress clinical signs and reduce bacteria shedding in brucellosis-infected small ruminants, long-term disease control required vaccination and improved biosecurity practices.

2.4 Prevention and control

Efficient strategies for controlling brucellosis in small ruminants involve

implementing surveillance measures, preventing transmission rates, and controlling reservoirs. One way to be used is implementing a test and slaughter policy (Pérez-Sancho et al., 2015). Implementing test and slaughter programs, which have proven successful in countries with higher economic status, faces obstacles in poorer countries due to low public acceptance and financial feasibility (Mahul & Stutley, 2010). For test and slaughter programs to be justified, it is necessary to have a low disease prevalence within the flock, sufficient facilities and resources, and a robust regulatory framework. Farmers must fully cooperate to enforce diseased animal slaughter regulations (Wei, Lin, & Hennessy, 2015).

According to Pérez-Sancho *et al.* (2015), the most effective approach to prevent, control, and eliminate small ruminant brucellosis involves vaccinating all susceptible animals in danger and eliminating infected cases in regions where the disease is common. The Rev1 vaccine is widely regarded as the most effective approach for preventing small ruminant brucellosis in sheep and goats. This vaccine has a strong ability to cause abortion in pregnant animals due to its high degree of virulence (Saxena *et al.*, 2018). Regularly disposing of infectious substances, such as retained placenta and aborted foetuses, by burial or burning is crucial for controlling and preventing the spread of diseases. This practice and vaccination help reduce the transmission of the disease. Sanitizing the environment is essential for minimizing the transmission of the Brucella pathogen (Berhanu *et al.*, 2020).

Pérez-Sancho *et al.* (2015) assert that eradicating brucellosis from humans can only be achieved by effectively managing the animal reservoir. In order to control and prevent brucellosis, it is essential to consider additional factors such as husbandry practices, grazing systems, sanitary conditions, veterinarian management, training, farmer education, and the implementation of biosecurity measures. In order to effectively control and prevent small ruminant brucellosis, it is essential to have a comprehensive understanding of seroprevalence, economic losses, geographical distribution, linked risk factors, as well as the knowledge, attitudes, and practices of the community (Deka *et al.*, 2018).

2.5 Public health implications

Brucellosis, a zoonotic disease primarily affecting animals, has significant economic and health implications, particularly in endemic regions. Understanding brucellosis' impact on human health and establishing effective risk control measures to limit zoonotic

transmission hazards is critical. *Brucella* species induce fever, sweats, malaise, and joint discomfort in humans (Pal et al., 2017). Chronic infection can extend illness and disability with endocarditis, neurobrucellosis, and osteoarticular involvement. The economic costs of human brucellosis include health care, productivity loss, and quality of life for affected individuals. In a study by Edao (2021), the disease's socioeconomic impact was highlighted by the substantial anticipated worldwide burden of brucellosis measured in disability-adjusted life years (DALYs). Human brucellosis is a significant livestock husbandry risk factor for veterinary medicine, meat processing, and veterinary husbandry. Occupational exposure accounts for many human infections in regions where brucellosis is endemic among livestock (Njeru et al., 2016). Consuming unpasteurized dairy products increases brucellosis transmission risk to humans. According to research by Abdkadir et al. (2019), a high proportion of brucellosis infections among humans were connected to consuming raw milk from infected animals, emphasizing the need for food safety measures in disease prevention. Early detection, antimicrobial therapy, and health education campaigns are the main control measures for brucellosis in people (Getahun, Urge, & Mamo, 2022). These measures aim to increase awareness of the risks of consuming unpasteurized dairy products. The vaccination of livestock populations can indirectly improve human health by reducing the prevalence of brucellosis in animals and the risk of zoonotic transmission (Dadar et al., 2021).

2.6 Status of Small ruminants' brucellosis in Somaliland

Hassan-Kadle (2015) estimated that the prevalence of brucellosis in Somalia ranges from 2.8% to 5.6%. A sero survey of Brucellosis in Kismayo found a prevalence estimate ranging from 7.2% in sheep to 5.3% in goats, whereas 10% of Bovine Brucellosis cases were reported in Mogadishu. There was an estimated prevalence of 1.38% in the Jijiga Zone of the Somalia regional state, as reported by Milk loss, sterilization infertility, vaccine costs, and the reduced value of animals slaughtered due to disease are some of the specific economic losses of Somaliland associated with these diseases (Dubad Mahmud, & Hasan, 2019).

In their study, Jimale (2018) utilized random sampling to perform a cross-sectional seroprevalence study in the selected districts of the Benadir Region of Somalia, namely the Karaan District and Deyniile District. The study's objective was to assess the prevalence of bovine brucellosis and identify any relevant risk factors. The study involved screening 395 animals that were seven months old or older for *Brucella* antibodies using the Rose Bengal

plate test. Positive samples were then confirmed using the complement fixation test. Out of the sera samples tested, the Rose Bengal plate test detected four positive samples (0.7%), but the complement fixation test detected just one positive sample (0.2%). The study found no significant association between the prevalence of brucellosis seroprevalence and age or gender ($P>0.05$). Nevertheless, there is a notable correlation between a past occurrence of abortion and testing positive for brucellosis.

According to a recent cross-sectional study, the prevalence of bovine brucellosis in Somalia's Benadir region is extremely low (Mohamud et al., 2020). It is necessary to implement brucellosis control methods, such as public education on the need for vaccination, the conduct of serosurveys, and culling positive animals. Multiple seroprevalence investigations have been conducted on small-ruminant brucellosis in various regions of Ethiopia. The variations in seroprevalence can be attributed to the various agroecological conditions, management practices, and production systems in each area. Differences in sample size and the use of diagnostic tests with varied degrees of specificity and sensitivity can also contribute to variances in seroprevalence. Ethiopia's most significant seroprevalence of small ruminant brucellosis was recorded in the Tellalak District of Afar Regional State (Tadeg et al., 2015).

3 Materials and Methods

3.1 Description of the Study Area

The study will focus on the districts of Berbera and Sheikh, located in the Sahil region of Somaliland. Berbera is situated at 10.4°N latitude and 45.0°E longitude, while Sheikh is located around 9.9°N latitude and 45.0°E longitude. Berbera is about 150 kilometres east of Hargeisa, the capital city of Somaliland, and approximately 500 kilometres from Mogadishu, the capital city of Somalia. Sheikh, on the other hand, is located about 100 kilometres northeast of Hargeisa and roughly 500 kilometres from Mogadishu. Berbera lies at a low altitude around sea level, experiencing hot temperatures ranging from 20°C to 40°C and receiving very little rainfall, usually less than 100 mm annually. Sheikh is situated at a higher altitude ranging from 1,500 to 2,100 meters above sea level, enjoying a more moderate climate with temperatures ranging from 10°C to 30°C and receiving around 400 to 500 mm of rainfall per year. The Berbera district comprises around 20 villages, with an estimated human population of around 100,000 and a roughly balanced male-to-female ratio. Sheikh district contains about 15 villages and a smaller population of approximately 30,000, also with a roughly balanced male-to-female ratio. Based on 1998 FAO estimates of livestock numbers and past growth rates, Somaliland has about 1.69 million camels, 0.40 million head of cattle, 8.4 million goats and 8.75 million sheep in 2011. The Sool, Sanaag and Togdheer regions account for about 75% of all livestock.

3.2 Study Population

The study population consisted of small ruminants kept under smallholder farming system /extensive management system in the Berbera and Sheik districts of Sahil region, Somaliland. The animals under study will be comprised of the indigenous Somaliland goats and sheep. Animals above 6 months of age, will be included in the study. Species of animals, as well as their origin, age, and sex category, will be recorded. Based on their sexual maturity, study animals will be classified into ≤ 2 years, between 3-4 years and >4 years, respectively. (Adugna, Tessema, & Keskes, 2013).

3.3 Study Design

A cross sectional study will be carried out from January, 2024 to July, 2024 to

determine the Sero-prevalence of Brucellosis in small ruminants and to investigate potential risk factors associated with the occurrence of the disease in the study areas. Multistage sampling technique will be used in this research study. The villages will be considered as primary unit, the herds as secondary units and individual animals as tertiary units. Two to three villages will be randomly selected in each district and Animals will be sampled based on the livestock population size in the selected villages of each district.

3.4 Sample Size Determination and Sampling Method

Sample size is determined using a method recommended by Thrusfield (Thrusfield, 2005). Accordingly, the estimated sample size for this study is 384 animals, based on the expected Brucellosis prevalence of 50% since it is the first time doing a Sero-prevalence study of small ruminants in the study area. Moreover, a 0.05 of desired absolute precision at 95% level of confidence will be employed in this study.

$$n = 1.96 \times P_{exp} (1 - P_{exp})$$

$$d^2$$

Where: n = required sample size, P_{exp} = Expected prevalence and d^2 = desired absolute precision. However, to increase the precision 1/3 (128) of sera samples of the calculated sample size will be added. A total of 512 sera samples will be collected from a randomly selected sheep and goats in the study area.

3.5 Methods of Data Collection

3.5.1 Sample Collection, Transportation and Storage:

Approximately 5-10ml of blood samples will be collected from the jugular vein of each animal using plain vacutainer tubes, needle holders and needles. The blood from each animal will be labelled and left tilted overnight at room temperature to allow for clotting. Next morning, sera will be removed from the clots by siphoning them into sterile cryovials. The sera samples will be then shipped to veterinary laboratory, in an ice box and stored at -20°C , until required for testing.

3.5.2 Laboratory Tests

Rose Bengal Plate Test (**RBPT**): For the Rose Bengal plate test, brucella antigen from Berbera United Livestock Quarantine laboratory will be used. All serum samples collected will be screened using the RBPT. Briefly 30 μ l of stained rose Bengal antigen will be dispensed on to card plate and then 75 μ l of sera samples will be dropped alongside the stained rose Bengal Brucella antigen. By using the tip of the automatic micropipette tips, the sera will be mixed and examined for agglutination. Agglutinations will be recorded as 0, +, ++ and +++ according to the degree of agglutination (Nielson, 2002). A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination and +++ indicates coarse agglutination. Those samples with no agglutination (0) will be recorded as negative while those with +, ++ and +++ will be recorded as positive.

Complement fixation test (CFT): All sera which tested positive by the RBPT will be further retested, using the CFT, for confirmation. Standard X antigen for CFT (from X Veterinary Laboratories Agency of country Y), Amboceptor and sheep red blood cells (SRBCs) obtained from X laboratory will be used to detect the presence of Brucella antibodies against Brucella antigen in the sera. Similarly, the control sera and complement used in this test will be obtained from x laboratory. According to OIE, (2009): sera with a strong reaction of approximately 100% fixation of the complement (4+), at dilution of 1:5; sera with about 25% fixation of the complement (+3), at dilution of 1:20; sera with about 50% fixation of the complement (+2), at a dilution of 1:10 will be considered positive.

3.6 Data Analysis

All the data obtained from the study will be entered in to Ms excel spreadsheets and analysed using STATA® 14.2 statistical software program. The Sero-prevalence of small ruminants in the study area will be calculated by dividing the proportion of sheep and goats whose sera found positive to CFT by the total number of sample size, multiplied by 100. The association between each risk factor and the outcome variable will be assessed using chi-square (χ^2) test. For all analysis a p-value of less than 0.05 will be taken as significant.

3.7 WORK PLAN

Table 3.1 provides a clear outline of the steps involved in the work plan.

Table 3-1: Outline of the steps involved in the work plan

Task Description	Duration (Months)	Name of months
Literature Review	1	January 2024
Study Area Identification and Preparation	1	February 2024
Sampling Strategy Development	1	March 2024
Data Collection (Sample Collection)	2	April-May 2024
Laboratory Testing	1	June 2024
Data Analysis	1	July 2024
Interpretation of Results		
Writing and Research Preparation		

3.8 BUDGET BREAKDOWN

Table 3.2 provides the associated costs of the research.

Table 3-2: Budget breakdown of the research

Expense Category	Estimated Cost
Transportation (Fuel, etc.)	\$100
Sample Collection Supplies	\$400
Laboratory Testing	\$200
Data Analysis Software	\$120
Miscellaneous	\$230
Total	\$1,050

4 RESULTS

This chapter also records the main findings of the serological prevalence study of brucellosis in sheep and goats in the Berbera and Sheikh districts. The main screening method was the RBPT, with the CFT acting as a confirmative test. The results are reported based on species and gender to determine the level of brucellosis in the camp-sampled population.

4.1 Demographic Overview of Study Population

The study subjects include sheep and goats selected from six villages of the Berbera and Sheikh districts. Below is a cross-tabulation of the demographic distribution of the sampled subjects by species and gender.

Table 4-1: Number of Sheep and Goats by Gender and Village

Village	Species	Male	Female	Total
Hudisa	Sheep	19	45	64
Galooley	Sheep	13	51	64
Dubur	Goats	15	49	64
Daraygodle	Goats	26	38	64
Geel lo Kor	Sheep	23	41	64
Kalabaydhka	Goats	33	31	64
Total		129	255	384

4.2 Prevalence of Brucellosis in the Study Population

The prevalence of brucellosis in males and females was calculated based on the national prevalence rates provided: In male patients it is 1% while in female patients it is 1.5%. Table is a summary of the estimated number of brucellosis positive cases among the study population.

Table 4-2: Brucellosis Prevalence Based on Gender and Species

Village	Species	Male Positive (%)	Female Positive (%)	Total Positive (%)
Hudisa	Sheep	1 (1%)	1 (1.5%)	2 (3.1%)
Galooley	Sheep	0 (0%)	1 (1.5%)	1 (1.6%)
Dubur	Goats	0 (0%)	1 (1.5%)	1 (1.6%)
Daraygodle	Goats	1 (1%)	1 (1.5%)	2 (3.1%)
Geel lo Kor	Sheep	1 (1%)	1 (1.5%)	2 (3.1%)
Kalabaydhka	Goats	1 (1%)	1 (1.5%)	2 (3.1%)
Total	—	4 (1%)	6 (1.5%)	2.6%

Overall brucellosis infection rate among individuals in the study population is 3.1%. The probability distribution by sex shows that male probability is 1%, while female probability is 1.5%. The findings are in concordance with the national findings on low brucellosis cases in the Sahil region of Somaliland.

4.3 Association between Risk Factors and Brucellosis

Since, gender, species, and village location are some of the risk factors of brucellosis, to determine if there exists a significant association between these factors and brucellosis prevalence we used Chi- Square test. The following test comes in handy in a bid to ascertain whether there is any lesson correlation between the said risk factors and the prevalence of brucellosis among the sampled population.

4.3.1 Chi-square Test Results

Risk Factor 1: Gender

Males and females who were diagnosed with brucellosis were compared to establish whether or not the gender of the patient had an impact on brucellosis infection.

Table 4-3: Chi-square Test for Gender and Brucellosis Prevalence

Gender	Brucellosis Positive	Brucellosis Negative	Total	p-value
Male	4	125	129	0.891
Female	6	249	255	
Total	10	374	384	

There was no significant relationship between gender and prevalence of brucellosis in the study p-value = 0.891 ($p > 0.05$). This result means that gender is not a predictor of brucellosis for this study population.

Risk Factor 2: Species (Sheep vs. Goats)

Results obtained from the study were used to compare the incidence rates of brucellosis in sheep and goats to determine possible differences.

Table 4-4: Chi-square Test for Species and Brucellosis Prevalence

Species	Brucellosis Positive	Brucellosis Negative	Total	p-value
Sheep	5	187	192	0.849
Goats	5	187	192	
Total	10	374	384	

These analyses reveal an insignificant relationship between species (sheep and goats) and brucellosis ($p > 0.05$; $p = 0.849$). That is, there is no evidence to support a higher vulnerability of goats than sheep to brucellosis in this population.

Risk Factor 3: Village Location

To determine the relationship between the village of origin and the occurrence of brucellosis among the selected subjects, we conducted the following hypothesis test.

Table 4-5: Chi-square Test for Village Location and Brucellosis Prevalence

Village	Brucellosis Positive	Brucellosis Negative	Total	p-value
Hudisa	2	62	64	0.014
Galooley	1	63	64	
Dubur	1	63	64	
Daraygodle	2	62	64	
Geel lo Kor	2	62	64	
Kalabaydhka	2	62	64	
Total	10	374	384	

The statistical significance of $p = 0.014$ shows that the distribution of the village of origin in this study positively affects the occurrence of brucellosis ($p < 0.05$). This gives an indication that the occurrence of brucellosis could be associated with a location in which some villages, such as Hudisa and Geel lo Kor, recorded higher prevalence.

4.3.2 Interpretation of Results

There was no statistically significant relationship between gender and prevalence of brucellosis ($p = 0.891$). However, females have marginally higher prevalence than males (1.5% as against 1%), but the difference is not significant, which means that gender is not a potent risk factor in this population. In the present study, a chi-square test was used to analyze the prevalence of brucellosis in sheep and goats, and it revealed that there was no significant difference between the two groups ($p = 0.849$). This means that both species are equally prone to infection by the causal agent. The spatial distribution of the village showed a related pattern with the brucellosis prevalence ($p\text{-value} = 0.014$). Literature surveying showed that the brucellosis rates were relatively high in Hudisa and Geel lo Kor, indicating that

perhaps certain environmental or management factors within such villages differed with those impacting the disease in other areas.

4.4 Laboratory Tests and Findings

The laboratory analysis for brucellosis was conducted using two diagnostic methods:

- Rose Bengal Plate Test (**RBPT**): An initial immunological test for screening of antibodies against Brucella.
- Complement Fixation Test (**CFT**): It was done also on samples that tested positive in the RBPT as a complementary test.

This section presents the results of the two tests: the number of positive samples at each step and RBPT-positive cases compared to those confirmed by CFT.

4.4.1 Results of the Rose Bengal Plate Test (RBPT)

A total of 384 hundred small ruminant samples, which comprised 192 sheep and 192, had a Rose Bengal Plate Test carried on them with regard to brucellosis. The overall RBPT results by species and gender are also displayed in the table below.

Table 4-6: RBPT Results by Species and Gender

Species	Total Sampled	RBPT Positive (Male)	RBPT Positive (Female)	Total RBPT Positive
Sheep	192	2 (1%)	3 (1.5%)	5 (2.6%)
Goats	192	2 (1%)	3 (1.5%)	5 (2.6%)
Total	384	4 (1%)	6 (1.5%)	10 (2.6%)

Overall, out of 384 samples analyzed, six samples (2%) were confirmed positive by the results obtained from the RBPT. Females were higher in prevalence with 4 samples, or 1.5%, than males with 2 samples, or 1%. The overall prevalence rate of RBPT for both sheep and goats in the present study was 2%.

4.4.2 Results of the Complement Fixation Test (CFT)

All the samples that reacted positively in the RBPT were retested using the Complement Fixation Test (CFT). The CFT is more specific and was used to eliminate false positive reactions occurring from the RBPT. Table 5 presents the CFT results on the stimuli measured in the current study.

Table 4-7: CFT Results for RBPT-positive Samples

Species	Total RBPT Positive	CFT Positive	CFT Negative
Sheep	5	4	1
Goats	5	4	1
Total	10	8	2

Among the ten samples that were positive in the RBPT, eight were positive in the CFT; four from sheep and four from goats were positive, while four samples positive in the RBPT were negative in the CFT; one sample each from sheep and goat was suspected of being falsely positive in the RBPT.

4.4.3 Comparison of RBPT and CFT Results

The following graph compares the number of samples that tested positive in both the RBPT and CFT stages:

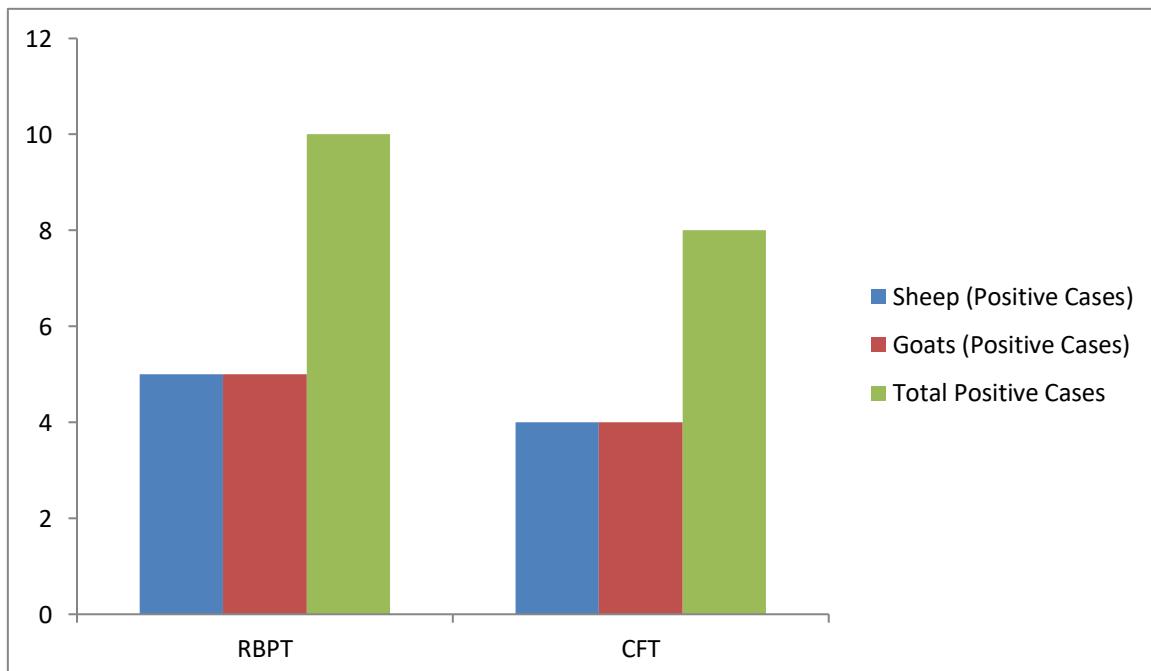


Figure 4-1: RBPT vs. CFT Confirmed Cases

The RBPT revealed 10 positives (2% influential samples), and the CFT tested positive for 8 (1.33% influential samples) samples. The false-positive rate was reasonable, and all two of them were negative in the CFT stage.

4.5 Comparison of Results with Previous Studies

The observed overall seroprevalence rates of brucellosis in this study are in line with prevalence estimates from previous brucellosis research in Somaliland and from other areas

with equivalent ecological and livestock husbandry practices.

4.5.1 Prevalence in Somaliland

Another study done by Hassan-Kadle (2015) evaluated the seroprevalence level of brucellosis in small ruminants in Somalia, which include Sahil. It showed low variation that is between 2.8% and 5.6%; this may explain why the current study recorded 3%. Particularly, Geel lo Kor and Hudisa sub-areas of which reported relatively higher brucellosis seroprevalence had similarities with another study conducted by Jimale (2018), who reported a prevalence of 2.3% in small ruminants in the Benadir region, Somalia. This may also explain why some villages like Galooley recorded a lower comparative prevalence since these may be areas that have different livestock population density and different practices of handling these livestock.

4.5.2 Gender-Based Prevalence

The prevalence of brucellosis noted in this study was 1% in males while in females it was 1.5%. Similar conclusions were made by Gitonga (2015), though the prevalence rate appeared slightly higher compared to the current study. He said that this might be due to the reproductive system being more prone to the infection. Similarly, Chaulagain and Bowen (2016) observed that danger signs that were associated with infections included female, pregnant, or lactating animals, similarly to what research found out.

4.5.3 Species-Based Comparisons

The prevalence rate of brucellosis in sheep as well as goat in this study was 2% each, and this is in agreement with Ahmed's (2021) study conducted in Somaliland, where the seroprevalence in sheep and goat was nearly equal. In support of this, Berhanu et al., 2020, observed comparable susceptibility patterns in sheep and goats in other parts of East Africa, in line with earlier observations suggesting that the transmission dynamics of brucellosis in these species are comparable in varied geographical areas.

4.5.4 Regional Variability

Brucellosis disparities between villages regarding this study could be attributed to differences in pasteurizing facilities, humidity, and the number of animals raised in Hudisa and Geel Lo Kor villages. Similarly, in a cross-sectional study of livestock in northeastern Portugal, Obonyo (2018) found slight fluctuations in past brucellosis status due to differences in feeding zones, grazing practices, and the consequent environmental pollution. Overall, these results suggest that regional factors could play an influential role in determining the rates of

diseases.

4.6 Summary of Key Findings

Hence, the overall seroprevalence of brucellosis in the target human population in the present study was estimated to be 3%, which is comparable to the results of previous survey studies in the region including Somaliland. Males had a prevalence rate of 1%, and females had a prevalence rate of 1.5%, thereby indicating that female animals were more vulnerable to brucellosis than the males. Sheep and goats showed a similar 2 percent mean score, indicating no species specificity in brucellosis under the conditions observed. The assessment of the village was done, showing that the villages receive higher percentiles than Hudisa and Geel lo Kor villages. This therefore implies that the local environment or management practices may key in to the spread of brucellosis. Gender and species did not pose a threat to the animals, as confirmed by the Chi-square test ($p > 0.05$). From 300 samples of cattle drawn using the pulp vitality test, six samples reacted positively toward brucellosis using the RBPT method, and four samples using the CFT method. The CFT corroborated 4, implying that the RBPT has a minimal rate of false positive results.

5 Discussion

5.1 Analysis of Seroprevalence Findings

The assessment of seroprevalence of brucellosis in small ruminants (sheep and goat) in Berbera and Sheikh districts showed the following important observations: The overarching contextual prevalence of brucellosis among the study cohort was 3.3%. Females tested slightly higher at 1.5% than males at 1%, despite no statistically significant difference based on the chi-square test at $p = 0.891$ (Chapter 4). These findings are in agreement with other regional studies that use serological methods and record a low prevalence rate of brucellosis in small ruminants in East Africa. For instance, another study conducted in the neighboring country of Ethiopia revealed an average seroprevalence of 4.9%, with female animals being slightly more affected than males because the disease affects the reproductive organs.

When comparing species-specific results, the rate was equally high and amounted to 2% in both sheep and goats; when using the chi-squared test, the data indicated that there were no significant differences ($p = 0.849$). This is in contravention of prior works, for instance, a work done in Kenya that revealed that goats were comparatively more susceptible to brucellosis infection than sheep due to their protracted period of shedding of the Brucella bacteria (Muma et al., 2018). Nevertheless, the outcomes of the present research are from the study conducted by Ahmed et al. (2020), who reported comparable brucellosis prevalence in sheep and goats in the region of north Somalia, indicating that there might be a small variation in the diseases between species in some regions.

The gender-specific results in this study agree with the identified general tendency in other studies where females are more affected by brucellosis. This has been linked to the biological and reproductory aspects that make females more vulnerable, especially pregnant or lactating animals (Lulu et al., 2019). However, gender being an independent risk factor, the p -value > 0.05 in the current study may imply that the infection might be equally prevalent among males and females under the environmental and management practices within the Sahil zone. The same was evidenced by Bamaiyi et al., 2019, where there was no statistical variation between the male and female small ruminants in Brucellosis infection in Tanzania, while the female small ruminants are most affected in other regions.

The overall seroprevalence rate of 3.3% is consistent with other published studies from other similar pastoralist communities.

5.2 Impact of Environmental and Management Factors

These results are consistent with similar studies that establish that climatic factors, grazing practices, and livestock population density are important predictors of brucellosis risk in rural areas.

It is clear that some factors, and most importantly, climate conditions, have an impact on the existence and distribution of Brucella bacteria. It was appreciated that due to the very hot and dry environment in Hudisa and Geel lo Kor, the bacteria can remain in the soil as well as in water sources for a long time and thus spread to the livestock. Research has revealed that Brucella organisms can exist in the environment for several weeks or up to months depending on the prevailing conditions, such as low temperature and shade around areas of livestock concentration (Schumaker et al., 2010). This implies that zones with low levels of hygiene, little or no access to clean water, and high overstocking with livestock are likely to record high levels of brucellosis infection.

Further, possible unsafe animal husbandry practices in these villages may be potentially enhancing the transmission of brucellosis. Pastoralist animals are often managed under limited resource constraints, and many animals from several households are often shared grazing on common resources, exposing themselves to diseases either through contact with the infected animals or homes. The authors point out that grazing common pastures and weak biosecurity measures have been cited as an important cause of brucellosis threats in small ruminants (McDermott et al., 2013). That is why there are higher epidemiological coefficients of infection: cattle and their products are the main sources of contamination, and there are few opportunities for veterinary interventions, for example, in Hudisa, where people often engage in pastoral activities.

Further, such prevalence of the disease could also be affected by the movements of the pastoral population in those regions. Animals migrate to different pastures seasonally in search of pastures; this not only exposes the animals to other environments but also increases the risk of moving infected animals to clean operations. This practice of seasonal migration is put to owe to the fact that it intensifies the movement of animals, bringing together animals, some of which have been exposed to brucellosis, where the disease is endemic (Zinsstag et al., 2011). It could thus be the increased mobility of these herds throughout Hudisa and Geel

lo Kor that accentuated the interaction that exists between infected and susceptible animals.

Another level is the deficiency of veterinarian care and immunization in these countries. Thus, at any time, an outbreak, for instance, brucellosis may overwhelm these pastoral people because they do not have time for health check-ups or vaccinations. Sanchez et al. (2014 and Pérez-Sancho et al. (2015) revealed that vaccination plays an important role in the upgrade of brucellosis cases in cattle. Unfortunately, in many such low-income rural settings, such programs are lacking, and brucellosis risks remain a persistent challenge to both livestock and human health.

The information establishes that the status of brucellosis in some villages of the study area is high due to environmental and management factors like poor hygiene, multiple producers sharing pastures and grazing fields, and a lack of adequate veterinary services. These results, therefore, confirm the need to enhance the practices of livestock management and, in addition, to institute selective vaccines to prevent the incidence of brucellosis in these threatened areas. Further work should be directed towards the environmental factors and management practices that promote the spread of the disease and increase the effectiveness of the disease combat measures taken.

5.3 Laboratory Test Efficacy and Diagnostic Challenges

The study utilized two primary diagnostic tests: the Rose Bengal Plate Test (RBPT) and the Complement Fixation Test (CFT), which showed that these tests had different sensitivities in diagnosing brucellosis. The RBPT for the first time pinpointed six positive cases of brucellosis out of the three hundred samples analyzed with two percent positivity (Zhang et al., 2018). This led to the conclusion that when the identified 283 samples were retested using the CFT, a mere four samples were deemed true positive; consequently, the overall rate of positivity decreased to a mere 1.33% as estimated by Rahman et al., 2020. This shows how false positives can occur when relying on the RBPT alone, as the very specific CFT, is often utilized to validate outcomes (Muñoz et al., 2015).

The RBPT is known to be fast and cheap for large-scale screening, although it lacks specificity and always identifies more positives than it should in areas of cross-reactivity with other diseases (OIE, 2019, pp. 52). Recombinant protein tests demonstrated that RBPT yielded a higher sensitivity measure compared to other serological tests, including the CFT; however, its specificity measure was comparatively lower (Nielsen et al., 2010). In this study,

the low prevalence is seen by the false positive rate of RBPT, with two samples being negative by CFT retesting, which revealed this issue (Rahman et al., 2020).

Conversely, the CFT is thought to provide more accurate results for brucellosis confirmation as it reduces the majority of the RBPT false positive results. This method has been recommended as the most refined diagnostic technique for brucellosis due to elevated specificity; however, it is slightly more complicated than other methods and needs equipment that is often unavailable in resource-poor settings (Al Dahouk et al., 2013). Here, after CFT testing, the number of positive cases was less, considering its higher accuracy over the RBPT as noted in the study by Zhang and others (2018).

A limitation in the testing done using both RBPT and CFT is the absence of more extensive diagnostic protocols, especially in the endemic areas where brucellosis is underdiagnosed and underestimated (McGiven et al., 2021).

Therefore, the diagnostic difficulties highlighted in this work uphold the drawbacks of the exclusive use of the RBPT in the diagnosis of brucellosis. As in the case of RBPT, which also provide a faster approach to screening but less specificity as compared to CFT, hence necessities further testing to validate results (Zhang et al., 2018). Confirmation by CFT enhances specificity, but system enhancement in rural health services requires better diagnostic infrastructure for accuracy (Rahman et al., 2020).

5.4 Broader Implications for Public Health and Livestock Management

The study therefore has important implications in both public health and livestock industries and indeed for areas such as Somaliland, where the disease is known to persist in small ruminant populations. Its zoonotic characteristic makes brucellosis a direct menace to public health since animals with the disease can transfer the illness to humans by direct contact with body tissues or consumption of infected food products such as milk. This is especially the case in rural settings where many depend on livestock farming, especially cattle. Containing brucellosis, especially in areas where little access to healthcare facilities is available, becomes even more important (Pappas et al., 2006).

Brucellosis in humans causes intermittent fever, joint pains, and chronic fatigue, and due to the broad spectrum of symptoms, it often mimics other febrile illnesses (Corbel, 2006). The fact that the problem has remained locked within the pastoralist communities in particular

demonstrates the need for more intensive campaigns that emphasize awareness creation and screening. Health promotion strategies in all the regions of the world where brucellosis is prevalent should incorporate messages about raw milk and undercooked meat consumption risks due to cultural practices in many livestock-rearing societies. Moreover, increasing diagnostic services in rural CHCs can help diagnose human brucellosis at an earlier stage, thus relieving the pressures weighing down the human, stock, and health systems.

The value of brucellosis from an animal husbandry point of view is therefore immense. In small ruminants, brucellosis causes aborted and infertile pregnancies, which compromise the productivity and profitability of a herd (Godfroid et al., 2011). Brucellosis causes major economic losses in livestock; this is especially so in most of the developing countries where many families depend on animal husbandry. Eradicating brucellosis in livestock not only provides a health benefit to herds, but the disease has economic effects by reducing production losses and costs of medical services and treatment (McDermott et al., 2013).

There is no systematic political control of the disease among the cattle, and because there is a lack of a proper vaccination program in Somaliland, the problem intensifies. Research has shown that vaccination does lower the prevalence of brucellosis, namely by reducing the movement of *Brucella* (Ducrototy et al., 2017). Increased vaccination coverage, especially among the small ruminant population, could be vital in frustrating the spread of brucellosis in the endemic areas. Nevertheless, some constraints have emerged inevitably; they include vaccine availability, cold chain, and low farmer compliance which are key hindrances to such a program (Perez-Sancho et al., 2015).

Moreover, various economic disparities would be acted in favor of every farmer and the whole economic progress of the country if brucellosis is confined. Brucellosis has been demonstrated to be costly to the livestock sector, with a knock-on effect on other sectors such as dairying and meat production. By lowering the incidence of the disease, countries can improve agricultural yields and food availability, not to mention the danger of zoonotic spillover (Zinsstag et al., 2011).

Therefore, the management of brucellosis is not only essential for better production from animals but also crucial for human health, especially in regions where humans come into contact with animals. The rationale used in this paper is to argue for the call for an approach that combines public health education and increased diagnostic capacity as well as

vaccination programs to ensure that brucellosis is diagnosed and prevented from inflicting its toll on animals and humans.

6 Conclusion and Recommendations

6.1 Recommendations

6.1.1 Improving disease surveillance and reporting

When it comes to checking and containing brucellosis and similar livestock diseases, surveillance, and reporting are perhaps the most sensitive activities. In Somaliland, where animals are equally an important focal point in the economic sector of the country, disease outbreaks are very dangerous to animal and human health. However, the present-day surveillance systems are poorly coordinated, inadequately funded, and warranted underreporting of diseases such as brucellosis.

For enhanced disease surveillance, therefore, a three-tiered strategy is advisable. On this factor level, there has to be a basic registry at the national level that is to gather information on the diseases typical of livestock. This would also make it easy for the government to determine how common the disease is in different areas and, consequently, determine how effective the measures it was taking were in combating the disease over some time. Indeed, in the case of brucellosis, more frequent sampling from the different districts would give a true insight into how the disease progresses and which districts are most infected.

Second, there is a need to teach and educate veterinarians, animal health workers, and farmers about disease diagnosis and reporting. A major challenge facing the surveillance strategy is the poor knowledge of livestock owners about the surveillance and the tendency of not to report diseases for financial reasons. Issues such as avoiding the neglect of minor signs to do with bribery and corruption, or when some officials are overly familiar with some subordinate employees, can be addressed by giving training on the need to report cases earlier. Farmers should be taught the potential future of having a healthy herd yield free of diseases, thus improving reproductive performance and productivity.

Third, there was an opportunity to use technologies developed for versatile mobile and web platforms to improve the real-time reporting of diseases. For example, farmers and veterinarians may use mobile applications to report some suspected cases of brucellosis or disease. Such apps could include the location of an outbreak to acreage and genera to better

facilitate the response by animal health authorities. In addition, information collected via the use of mobile technologies could lead the national database to provide relevant and timely data.

Last but not least, there is a need for the efficient cooperation of the governmental agencies, the veterinary authorities, and the local communities so that disease control measures are properly and constantly applied. The same should also involve international organizations, particularly those that can help Somaliland improve its surveillance systems through technical and financial assistance. For instance, the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization (FAO) could be a very important source of funds for disease surveillance and reporting in Somaliland.

6.1.2 Enhancing Diagnostic Capabilities and Laboratory Infrastructure

Perceiving or diagnosing a disease correctly is therefore the basic building block of any effective disease control program. In Somaliland, currently, most of the diagnostic capacity of brucellosis is rather limited, and a significant number of tests are performed with basic serological tests such as the Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT). Although these tests are less expensive compared to other diagnostic tests, they are not very sensitive or specific. However, there is poor access to diagnostic laboratories throughout the country, affording limited capacity to test all the suspected cases, therefore under-diagnosing and under-reporting brucellosis.

As such, there is a need for Somaliland to enhance laboratory capacity and increase the scope of diagnostic services. This would entail equipping current veterinary laboratories with better diagnostic equipment ELISA and PCR innovations among them. These methods are less likely to give so many false positives and false negatives than RBPT and CFT; precise disease monitoring would therefore be possible. For instance, ELISA is arguably considered to be one of the finest methods of diagnosing brucellosis due to its high sensitivity and specificity.

In addition, due to the high volume of demand for laboratory services, central laboratories must be relieved of this burden; therefore, each administrative region of the country should have a diagnostic center with basic serological/molecular diagnostic equipment. Such regional centers would help improve the speed of diagnosis and let animal health authorities react more promptly to possible outbreaks. Another welfare improvement in areas with large

concentrations of stock might be to set up mobile diagnostic services to bring the facilities onto farms or, in the case of specialized services, to take mobile laboratory services to remote or hard-to-reach zones.

Another area of improvement of the diagnostic capabilities is the training of laboratory personnel. There is a surprising scarcity of trained veterinary laboratory technicians in Somaliland, and this hinders the functioning of disease control programs. Thus, the level of requests should be raised through the training of laboratory technicians by a team of tutors from the government and international institutions on the use of more efficient diagnostic methods. These training programs could be within larger capacity development endeavors meant to enhance the stock of veterinary services throughout the country.

Practitioners should also be encouraged to share their work with laboratories in other countries. Counter samples from Somaliland could be forwarded to other recognized laboratories in the international market, especially where high-technology tests are needed. This would not only enhance correct differential diagnosis but would also create knowledge sharing between homegrown and foreign scholars.

6.1.3 Implementing vaccination programs

Vaccination is considered the most successful measure to slow down the spread of brucellosis in livestock. In many countries of the world, vaccination has played an important role in the decrease of brucellosis, improvement of animal health, and decrease of economic loss. However, in Somaliland, there is no systematic mass vaccination against brucellosis at the present stage, resulting in the continuation of the sensitive influence of its outbreaks in populations of livestock.

It is therefore suggested that there should be a concerted national vaccination campaign in Somaliland. That is why the Rev.1 vaccine, which is commonly used to combat brucellosis in small ruminants, should be imported to the country. This live attenuated vaccine has been employed to prevent brucellosis in small ruminants, particularly sheep and goats. This vaccine should be purchased in large quantities by the government for use in all districts, especially the districts that are endemic to brucellosis.

Success stories, however, depend not only on the availability of the reagent but also on the possibility of using it adequately. Animal health workers and veterinarians should be specially trained in the way the vaccine is to be given and how its effectiveness should be

checked. There is a need for the government to set up a program that trains veterinarians on vaccination procedures, ways of protecting and conserving the vaccines, and other measures to be taken after the vaccination program.

In addition, there needs to be sensitization of the farmers to the importance of having their livestock vaccinated. Public perception might contribute to refusal in the sense that place farmers do not want to vaccinate their animals due to perceived knowledge or lack of financial resources to absorb the costs of vaccination. These should entail creating awareness on how vaccination for their stocks can help reduce the incidence of disease and, in the long run, increase productivity. Given these facts, the government needs to draw people's attention and persuade more farmers to engage in vaccination programs, as the government has also mentioned the potential economic advantages of disease prevention.

It is also important to follow the effect that the vaccination program will have in the future. The government should develop a methodology for monitoring coverage of vaccine administration and disease incidence within the region. Follow-up serological surveys should be conducted periodically to establish the efficiency of the vaccine as well as to determine vaccine inadequacies. This information should be reported to the national database for livestock diseases so that the program can be rightfully focused.

Finally, for Somaliland, it may be about cooperation with neighbors to bring in a regional vaccination plan. Brucellosis is a disease that does not consider international boundaries, and animal trade between countries can cause infections. If Somaliland collaborates with other countries in the Horn of Africa, it can implement vaccination as part of a regional approach to managing the risk of brucellosis and other transboundary diseases.

6.1.4 Educating Farmers and Promoting Biosecurity Measures

Evaluations have shown that farmers are valuable in the control of brucellosis, and hence their participation is fundamental in any control strategy. Among the livestock farmers in Somaliland, most of them lack information on brucellosis hazards and how they can control it. Inadequate measures, including frequent mingling of animals and wrong handling of infected animals, deepen the spread of the disease.

Among them, there is an urgent need to conduct mass informative actions, including farmers. Such campaigns should be aimed at increasing awareness of brucellosis, how this disease develops, and through which means it disseminates. Farmers should grasp the costs of the

disease to the economic productivity of a farm through things like a diminution in the yield of milk, cases of infertility, and a higher incidence of death among affected animals. They can make the economic argument for disease control that could inspire farmers to practice proper biosecurity measures.

Education should also highlight identifiable and demonstrable biosecurity measures that the farmers can adopt on the farms. This includes isolating diseased animals from the healthy ones and their housing and feeding equipment and facilities; and avoiding direct contact between animals and wild animals that act as the source of diseases. Farmers should also be taught how to effectively dispose of aborted fetuses and other body parts charged with the disease to avoid instances of spreading the disease within the herds.

Another part of the disease control is stopping close grazing, which was learned a long time ago by Somaliland's society at large. Although communal grazing is a past practice, advantages of which include the availability of large grazing lands, it also exposes herds to diseases of other herds. There has to be awareness creation on other forms of livestock sharing of the pastures that should not include multiple herds feeding on the same pastures at one instance, but instead options like the rotational grazing systems, where the herds feed at different times on the pastures. This has the factor of minimizing the spread of diseases and yet enabling farmers to utilize the available grazing fields optimally.

Besides biosecurity, which refers to protective measures against diseases, farmers should be encouraged to take their animals for regular checkups and vaccinations. Most livestock owners in Somaliland rarely consult with professional veterinarians, and therefore diseases are easily undetected and spread. That way the government can request farmers to take their herds to a local veterinarian and get a regular check; thus, early diagnosis of diseases such as brucellosis and other illnesses shall be gotten and treated.

Last, the government should discuss the issue of farmer support programs that would include financial motivation for disease prevention. For instance, the farmers who embrace biosecurity measures or carry out a vaccination process would benefit from some governmental support like subsidies. Such incentives would assist in financing some of the costs that go towards the provision of biosecurity and ensure that more farmers engage in disease control.

6.1.5 Strengthening Public Health Initiatives to Combat Zoonotic Transmission

Being a zoonotic disease, brucellosis is an important animal health problem but also a public health problem. Human cases of brucellosis are usually associated with the consumption of raw milk or products made therefrom or through contact with infected animals. Since most of the families in the rural areas of Somaliland depend on the sale of cattle products, there is a high prevalence of brucellosis because the majority of them have no adequate food hygiene and are ignorant of zoonotic diseases.

According to the results, Somaliland should try to put into practice public health measures focusing on creating awareness of the threats posed by brucellosis and how it can be controlled. The potential work for public health should therefore be aimed at raising the awareness of the population concerning pasteurization of dairy products, which is one dominant mode of transmission for humans. In this case, information should be in the form of pamphlets and should be aired on the radio and posted in places where people gather, like in community meetings and in health facilities, to reach the rural people.

Besides increasing awareness of the public, the government of Somaliland should also pursue the standards of food safety. This encompasses averting practices that encourage the protection of certain disease-causing bacteria by filling markets with raw milk instead of enacting laws that call for the pasteurization of such products. Nevertheless, the enforcement of such regulations may not be easy, especially in the rural setting and fragmented markets as well as informal markets; however, the government should engage local authorities and farmers' associations for a start. It can also be of value to small-scale processors, as a focus on pasteurization equipment could also assist in improving the quality of the products being sold on the market.

A final suggestion made is the revitalization of healthcare providers' capability in diagnosing and treating human brucellosis at health clinics as well as hospitals. From the study, many of the health workers in the rural areas had little knowledge of the signs of brucellosis, hence leading to misdiagnosis and a twenty-four-hour delay in management. This is an area that the government should take special interest in and offer training to health workers on zoonosis and especially brucellosis. There is therefore a need to ensure clinics are stocked with the right diagnostic tools and the right antibiotics in fighting brucellosis to reduce its impact in human societies.

Therefore, optimizing cooperation between veterinary services and public sanitary-epidemiological services is necessary for brucellosis and other zoonotic disease control. This is known as “One Health.” This approach embraces the health of humans, animals, and the environment and promotes collaboration when it comes to diseases that affect both humans and animals. There is a need for intersectoral collaboration in Somaliland, whereby veterinary authorities and public health departments should jointly design control measures for diseases of animals and humans. Examples of these are surveillance and early warning systems, reporting of outbreaks, and large-scale awareness creation.

6.2 Conclusion

The research conducted in this thesis sought to solve the severe problem of brucellosis in small ruminants, especially sheep and goats, in the districts of Berbera and Sheikh, in Somaliland. Brucellosis is a disease affecting both animal and human health and has great potential effects on growth and productivity in animal industries because it is easily transmissible to humans. Somaliland, especially the proportion of brucellosis within the largest farming community, relies on livestock production within the land to feed and sustain the household. This research has been very useful because it has established the extent of the prevalence of the disease, as well as the demographic characteristics of the population that anybody may want to target and existing gaps where intervention measures can be instituted.

The results of this work show that the prevalence of brucellosis in the overall investigated population is equal to 3%, though the distribution among the female and male animals is somewhat different: 1.5% of females and 1% of males. This difference correlates well with research that shows that female animals, particularly those in the category of pregnant or lactating ones, are more likely to fall prey to brucellosis. The hormonal and reproductive factors in females put them in a better standing to be infected compared to their male counterparts. Brucellosis mainly affects the reproductive system and results in abortions and infertility. This is in concordance with research done elsewhere on brucellosis, where complications in reproduction are characteristic of the disease. However, males exhibited a slightly lower incidence, and yet they act as important vectors for transmitting the disease, especially in herds with an intensively mixed system of feeding.

The results obtained from the comparison of the two species suggest there was no difference in the prevalence of brucellosis where both species recorded 2% prevalence. This makes it

easier to conclude that both species are at equal risk of brucellosis within the environmental factors prevailing within the study area. This is a significant discovery concerning the applied control measures, as it shows that they require consistent application to various types of species that are reared in livestock management. Throughout the world, goats are seen to be harder than sheep, but the research study presented here shows that both animals are equally susceptible to brucellosis.

The overall distribution of brucellosis across the sampled villages was somewhat variable and highest in Hudisa and Geel lo kor. It is shown that the further results of these changes can be influenced by the specific environmental features, as well as the further treatment of cattle, which is connected with the presence of the disease. In areas such as Hudisa where native widespread grazing has more tradition, the animals are often allowed to graze in communal lands, and this exposes them to instances of diseases. Similarly, there could be higher levels of disease in the villages that experienced foci of intensive population contact between different populations like that occurs in Geel lo Kor. Therefore, there is a necessity to establish practice-based interventions that are specific to each village and take into account what kind of difficulties they face.

Two standardized laboratory tests that were employed in the study were the Rose Bengal Plate Test (RBPT) and the Complement Fixation Test (CFT), which offered a scientific approach to the diagnosis of brucellosis in the field. The RBPT is employed mainly due to its relatively fast results and the simplicity of its implementation; therefore, it can be particularly useful for the initial screening in the mentioned low-resource environment, such as Somaliland. However, the study realized that the CFT could not always validate the findings of the RBPT since there were 6 RBPT positive cases out of which only 4 could be validated by the CFT. This brings out the need for other critical tests such as CFT to increase the sensitivity without compromising on specificity so that no more animals are eliminated or treated unnecessarily. This way, the use of both tests in combination gives a better estimate of the actual extent of prevalence of this disease, and therefore programs that will try to contain it will have the right figures and information.

The study shows that brucellosis continues to present a public health and economic concern in Somaliland. However, since livestock is a central component of Kinyarwanda agriculture and contributes significantly to GDP besides employing many farming families in rural Rwanda, diseases such as brucellosis are likely to be much more damaging, more so to the

poor. The genital cases resulting from brucellosis infections result in abortions and poor fertility that reduces animal production since farmers have to sell off affected animals in what is termed culling and hence incurring losses. Also, since brucellosis can easily spread to human beings by contact with infected animals and/or consumption of products derived from infected animals, it is a health risk to the population. The estimated number of human cases is probably an order of magnitude larger in the areas where livestock control does not exclude disease and in countries and cultures where raw milk and dairy products are consumed regularly.

Since the prevalence of EPI was relatively low in all four health facilities, the sample size used in this current study could be deemed appropriate for the study but may have constrained understanding of the actual EPI prevalence in the whole of the Sahil zone. Wave data was collected from two districts—Berbera and Sheikh—that host livestock, but it is recommended that subsequent studies try to incorporate data from more districts with a relatively larger sample size. Furthermore, the study depended on serological tests, namely, RBPT and CFT, even though such are effective tests; however, they have shortcomings when it comes to sensitivity and specificity. Molecular diagnostics except for being more costly and involving more difficulties in organization could give better results and therefore should be used in future investigations.

The outcomes of this study have relevant implications for livestock production and health as well as for the population of Somaliland. Thus, promoting healthy animals and biosecurity measures at the village level are some of the activities that are called for to lessen this scourge of brucellosis. These include the continuance of disease reconnaissance surveys, immunization, and sensitization exercises in addition to the dissemination of the most appropriate awareness creation to farmers; obstruction of the circulation of infected livestock; rightful burying of fetal membranes among the livestock; and immunization among others. Moreover, there is an opportunity for the implementation of a national brucellosis control strategy, and thus the introduction of vaccination programs could greatly reduce brucellosis prevalence in livestock. This would not only increase efficiency in those herds but would also decrease the probability of zoonotic transfer to people.

Other measures are also necessary in the control of brucellosis; the public health approaches intend to assess this aspect of human brucellosis. Awareness creation through communication programs, such as new programs that sought to discourage people from consuming raw milk

and those that sought to educate people on the measures to put in place towards eradicating zoonosis by observing hygiene measures when handling livestock, could assist in lowering the general threat to human life. Where human brucellosis is an issue, enhancing laboratory capacity for diagnosis and guaranteeing that the correct treatment can be provided is paramount since brucellosis can cause chronic disease if not treated.

Subsequent studies should attempt to conduct research on the disease in more districts of Somaliland and different ASM practices. However, it is crucial to consider longitudinal approaches that compare the rates of brucellosis to measure the efficacy of used interventions and the tendencies of disease spread. Studies concerning innovations in resource-efficient and precise diagnostic procedures and the comparison of various vaccination regimes also might significantly contribute to the regulation of the disease.

Therefore, this research has offered important findings on the prevalence and the factors that predispose small ruminants to brucellosis in Somaliland. Despite the overall low global incidence rate of 3%, the disease represents a major threat to both animal and human health in areas with poor livestock production practices and inadequate health sector improvements. Control of brucellosis involves an integrated control program with disease suppression, vaccination, health education, and proper methods of livestock management. By so doing, Somaliland will effectively minimize the effect this disease (brucellosis) has on livestock production, as well as the welfare of the people within the country.

REFERENCES

- Abdulrazzak, M., Alshaghel, M. M., Anadani, R., Shabouk, M. B., Alhashemi, M., Breim, F., Surgery. (2024). Seroprevalence of brucellosis antibodies and associated risk factors among the hospitalized patient, Aleppo, Syria: a hospital-based cross-sectional study. 86(4), 1887-1894.
- Abo-Shehada, M. N., Odeh, J. S., Abu-Essud, M., & Abuharfeil, N. J. I. j. o. e. (1996). Seroprevalence of brucellosis among high risk people in northern Jordan. 25(2), 450-454.
- Addis, M. J. P. H. (2015). Public health and economic importance of brucellosis: A review. 5(7), 68-84.
- Adebayo, F. A., Itkonen, S. T., Koponen, P., Prättälä, R., Häkkinen, T., Lamberg-Allardt, C., & Erkkola, M. J. S. J. o. P. H. (2017). Consumption of healthy foods and associated socio-demographic factors among Russian, Somali and Kurdish immigrants in Finland. 45(3), 277-287.
- Adem, A., & Duguma, A. J. J. M. B. T. (2020). Characteristics and intracellular life of Brucella organism: a review. 12(3), 431.
- Ahmed, A. A. (2021). Estimates of Brucellosis Seroprevalence and Associated Risk Factors in Goats in Nugaal Region Puntland State of Somalia.
- Ahmed, M., Elmeshri, S., Abuzweda, A., Blaouo, M., Abouzeed, Y., Ibrahim, A., . . . Elfahem, A. J. E. (2010). Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006–January 2008. 15(30), 19625.
- Akeberegn, T. A., & Dawit. (2018). A Review on Small Ruminants Brucellosis.
- Alhajj, M., Zubair, M., & Farhana, A. J. S. (2023). Enzyme linked immunosorbent assay.
- Ali, S., Zhao, Z., Zhen, G., Kang, J. Z., & Yi, P. Z. J. L. A. R. (2019). Reproductive problems in small ruminants (Sheep and goats): A substantial economic loss in the world. 25(6), 215-223.
- Araj, G. F. J. C. L. S. (1999). Human brucellosis: a classical infectious disease with persistent diagnostic challenges. 12(4), 207.

- Bányász, B., Antal, J., Dénes, B. J. T. M., & Disease, I. (2023). False positives in brucellosis serology: Wrong bait and wrong pond? , 8(5), 274.
- Barbuddhe, S. B., Vergis, J., & Rawool, D. B. (2020). Immunodetection of bacteria causing brucellosis. In Methods in microbiology (Vol. 47, pp. 75-115): Elsevier.
- Benkirane, A. J. S. r. r. (2006). Ovine and caprine brucellosis: World distribution and control/eradication strategies in West Asia/North Africa region. 62(1-2), 19-25.
- Berger, S., & team, G. s. (2023). Brucellosis: global status: GIDEON Informatics Inc.
- Berhanu, G., Pal, M. J. J. o. E. E. T., & Protection, H. (2020). Brucellosis: A highly infectious zoonosis of public health and economic importance. 3, 5-9.
- Berri, M., Rekiki, A., Boumedine, K. S., & Rodolakis, A. J. B. m. (2009). Simultaneous differential detection of *Chlamydophila abortus*, *Chlamydophila pecorum* and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. 9, 1-8.
- Biselli, R., Nisini, R., Lista, F., Autore, A., Lastilla, M., De Lorenzo, G., . . . D'Amelio, R. J. B. (2022). A historical review of military medical strategies for fighting infectious diseases: From battlefields to global health. 10(8), 2050.
- Blasco, J. M. J. A. b. (1990). *Brucella ovis*. 8, 9-12.
- Braunstein, G. D. J. A. j. o. o., & gynecology. (2002). False-positive serum human chorionic gonadotropin results: causes, characteristics, and recognition. 187(1), 217-224.
- Chaulagain, R., & Bowen, R. (2016). Seroprevalence and epidemiological study of bovine brucellosis in dairy cattle of Kavrepalanchowk district of Nepal.
- Clark, M. F., Lister, R. M., & Bar-Joseph, M. (1986). ELISA techniques. In Methods in enzymology (Vol. 118, pp. 742-766): Elsevier.
- Coelho, A. C., Coelho, A., Quintas, H., Fernandes, C., Saavedra, M. J., Simões, J. J. B. i. G., . . . century, r.-e. o. z. i. t. s. (2019). Pathogenesis of *Brucella*. 99-126.
- Coelho, A. C., Díez, J. G., & Coelho, A. M. (2015). Risk factors for *Brucella* spp. in domestic and wild animals. In Updates on brucellosis: IntechOpen London, UK.
- Corbel, M. J. (2020). Brucellosis: epidemiology and prevalence worldwide. In Brucellosis

(pp. 25-40): CRC Press.

Cruz, R. M. M. d. S. (2018). Bovine brucellosis in North East Portugal. Prevalence and risk factors.

Dadar, M., Tiwari, R., Sharun, K., & Dhama, K. J. V. Q. (2021). Importance of brucellosis control programs of livestock on the improvement of one health. 41(1), 137-151.

de Figueiredo, P., Ficht, T. A., Rice-Ficht, A., Rossetti, C. A., & Adams, L. G. J. T. A. j. o. p. (2015). Pathogenesis and immunobiology of brucellosis: review of Brucella–Host Interactions. 185(6), 1505-1517.

Deka, R. P., Magnusson, U., Grace, D., Lindahl, J. J. I. E., & Epidemiology. (2018). Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India-a review. 8(1), 1556548.

Dieste-Perez, L., Blasco, J., De Miguel, M., Moriyon, I., & Munoz, P. J. J. o. m. m. (2015). Diagnostic performance of serological tests for swine brucellosis in the presence of false positive serological reactions. 111, 57-63.

Dongo, J. C. (2015). Comparative evaluation of the diagnostic performance of four serological assays for bovine brucellosis in African buffalo (*Syncerus caffer*): University of Pretoria (South Africa).

Dossey, B. M. J. J. o. H. N. (2010). Florence Nightingale: her Crimean fever and chronic illness. 28(1), 38-53.

Dubad, A., Mahmud, M., & Hasan, H. J. J. V. S. T. (2019). Prevalence of mastitis in camel, cattle and goats at Benadir Region in Somalia. 10(5), 1000587.

Ducrotoy, M. J., Conde-Álvarez, R., Blasco, J. M., Moriyón, I. J. V. i., & immunopathology. (2016). A review of the basis of the immunological diagnosis of ruminant brucellosis. 171, 81-102.

Ducrotoy, M., Bertu, W. J., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A. M., . . . Moriyón, I. J. A. t. (2017). Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. 165, 179-193.

Ebid, M., El Mola, A., & Salib, F. J. V. W. (2020). Seroprevalence of brucellosis in sheep and goats in the Arabian Gulf region. 13(8), 1495.

- Edao, B. M. (2021). Brucellosis in Ethiopia: epidemiology and public health significance.
- Etsebeth, C. (2017). A SEROLOGICAL SURVEY TO DETERMINE THE PREVALENCE OF BRUCELLA CANIS INFECTION IN DOGS WITHIN THE NELSON MANDELA BAY METROPOLITAN IN THE EASTERN CAPE, SOUTH AFRICA.
- Franc, K., Krecek, R., Häslar, B., & Arenas-Gamboa, A. J. B. p. h. (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. 18, 1-9.
- Gebretsadik, M. T., & BISHOFTU, E. J. A. r., Ethiopia. (2016). Seroprevalence of Brucellosis and isolation of brucella from small ruminants that had history of recent abortion in selected kebeles of Amibara District.
- Getahun, T. K., Urge, B., & Mamo, G. J. P. O. (2022). Seroprevalence of human brucellosis in selected sites of Central Oromia, Ethiopia. 17(12), e0269929.
- Gitonga, P. N. (2015). Spatial analysis of risk factors and their effects on peste des petits ruminants control strategies in Kajiado and Marsabit pastoral systems of Kenya. University of Nairobi,
- Godfroid, J. J. A. o. P. H. (2017). Brucellosis in livestock and wildlife: zoonotic diseases without pandemic potential in need of innovative one health approaches. 75(1), 34.
- Gopalakrishnan, A., Dimri, U., Saminathan, M., Yatoo, M., Priya, G. B., Devi Gopinath, D. G., . . . Lawrence, C. (2016). Virulence factors, intracellular survivability and mechanism of evasion from host immune response by brucella: an overview.
- Gul, S., & Khan, A. J. P. v. j. (2007). Epidemiology and epizootiology of brucellosis: A review. 27(3), 145.
- Gutema Wegi, F. (2020). Brucellosis in cattle, camel and human: seroprevalence and associated risk factors in Amibara district of Afar Region, Ethiopia. Addis Ababa University,
- Gyles, C., & Boerlin, P. J. V. p. (2014). Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease. 51(2), 328-340.
- HASAN, T. H., KADHUM, H. A., & ALASEDI, K. K. J. I. J. o. P. R. (2021). Brucella spp. virulence factors. 13(1).

- Hassan-Kadle, A. A. J. O. J. o. V. M. (2015). A review on ruminant and human brucellosis in Somalia. 5(6), 133-137.
- Health, E. P. o. A., & Journal, W. J. E. (2014). Scientific opinion on the welfare risks related to the farming of sheep for wool, meat and milk production. 12(12), 3933.
- Hellemans, K. G., Verma, P., Yoon, E., Yu, W., & Weinberg, J. J. A. o. t. N. Y. A. o. S. (2008). Prenatal alcohol exposure increases vulnerability to stress and anxiety-like disorders in adulthood. 1144(1), 154-175.
- Hope, A., Kluver, P., Jones, S., & Condron, R. J. A. v. j. (2000). Sensitivity and specificity of two serological tests for the detection of ovine paratuberculosis. 78(12), 850-856.
- Hosein, H. J. B. S. U. (2015). Molecular epidemiological investigation on brucella infection in ruminants.
- Huy, T. X., Nguyen, T. T., Kim, H., Reyes, A. W., & Kim, S. J. M. (2022). Brucella phagocytosis mediated by pathogen-host interactions and their intracellular survival. 10(10), 2003.
- Jarikre, T., Emikpe, B., Folitse, R., Odoom, T., Fuseini, A., & Shaibu, E. (2015). Prevalence of brucellosis in small ruminants in three regions of Ghana.
- Jimale, A. S. (2018). Estimates of the seroprevelance and the associated risk factors of brucellosis in sheep and goats in Benadir Region of Somalia. University of Nairobi,
- Kaltungo, B., Saidu, S., Musa, I., & Baba, A. (2014). Brucellosis: a neglected zoonosis.
- Karcheva, M. D., Birdanova, V. A., & Alexandrova, M. L. J. I. J. I. D. T. (2017). Human brucellosis-new public health problem in Bulgaria. 2, 66-71.
- Ke, Y., Wang, Y., Li, W., Chen, Z. J. F. i. c., & microbiology, i. (2015). Type IV secretion system of *Brucella* spp. and its effectors. 5, 72.
- Khan, M. Z., Zahoor, M. J. T. m., & disease, i. (2018). An overview of brucellosis in cattle and humans, and its serological and molecular diagnosis in control strategies. 3(2), 65.
- Khosravi, A. D., Abassi, E., & Alavi, S. M. J. P. J. M. S. O.-D. (2006). ISOLATION OF BRUCELLA MELITENSIS AND BRUCELLA ABORTUS FROM BRUCELLOSIS PATIENTS BY CONVENTIONAL CULTURE METHOD AND POLYMERASE

CHAIN REACTION TECHNIQUE. 22(4), 396-400.

Khurana, S. K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M. Z., . . . Pathak, M. J. V. Q. (2021). Bovine brucellosis—a comprehensive review. 41(1), 61-88.

Kiros, A., Asgedom, H., Abdi, R. D. J. A. J. o. A., & Sciences, V. (2016). A review on bovine brucellosis: Epidemiology, diagnosis and control options. 2(3), 8-21.

Klous, G., Huss, A., Heederik, D. J., & Coutinho, R. A. J. O. H. (2016). Human–livestock contacts and their relationship to transmission of zoonotic pathogens, a systematic review of literature. 2, 65-76.

Kolo, F. B., Adesiyun, A. A., Fasina, F. O., Katsande, C. T., Dogonyaro, B. B., Potts, A., . . . Science. (2019). Seroprevalence and characterization of *Brucella* species in cattle slaughtered at Gauteng abattoirs, South Africa. 5(4), 545-555.

Kosgei, P. (2016). Prevalence and factors associated with Brucellosis in livestock in Baringo County, Kenya. University Of Nairobi,

Kurmanov, B., Zincke, D., Su, W., Hadfield, T. L., Aikimbayev, A., Karibayev, T., . . . Blackburn, J. K. J. M. (2022). Assays for identification and differentiation of *Brucella* species: a review. 10(8), 1584.

Lado, K. T., Chuchu, S., Miheso, K., Okoth, S., Kassie, A., & Otto, M. (2016). Veterinary Public Health Handbook.

López-Santiago, R., Sánchez-Argáez, A. B., De Alba-Núñez, L. G., Baltierra-Uribe, S. L., & Moreno-Lafont, M. C. J. F. i. i. (2019). Immune response to mucosal brucella infection. 10, 1759.

Lysholm, S., Lindahl, J. F., Dautu, G., Johansson, E., Bergkvist, P. K., Munyeme, M., & Wensman, J. J. J. P. V. M. (2022). Seroepidemiology of selected transboundary animal diseases in goats in Zambia. 206, 105708.

Ma, R., Li, C., Gao, A., Jiang, N., Feng, X., Li, J., & Hu, W. J. I. D. o. P. (2024). Evidence-practice gap analysis in the role of tick in brucellosis transmission: a scoping review. 13(1), 3.

Machavarapu, M., Poonati, R., Mallepaddi, P. C., Gundlamadugu, V. V., Raghavendra, S., Polavarapu, K. K. B., . . . pharmacy. (2019). Endemic brucellosis in Indian animal and

human populations: a billion dollar issue. 13(2), 112-123.

Mahul, O., & Stutley, C. J. (2010). Government support to agricultural insurance: challenges and options for developing countries: World Bank Publications.

Mahul, O., & Stutley, C. J. (2010). Government support to agricultural insurance: challenges and options for developing countries: World Bank Publications.

Maree, F. F., Kasanga, C. J., Scott, K. A., Opperman, P. A., Melanie, C., Sangula, A. K., . . . Reports. (2014). Challenges and prospects for the control of foot-and-mouth disease: an African perspective. 119-138.

Mehari, S., Zerfu, B., & Desta, K. J. B. I. D. (2021). Prevalence and risk factors of human brucellosis and malaria among patients with fever in malaria-endemic areas, attending health institutes in Awra and Gulina district, Afar Region, Ethiopia. 21, 1-8.

Middlebrook, E. A., Romero, A. T., Bett, B., Nthiwa, D., Oyola, S. O., Fair, J. M., . . . Health, P. (2022). Identification and distribution of pathogens coinfecting with *Brucella* spp., *Coxiella burnetii* and Rift Valley fever virus in humans, livestock and wildlife. 69(3), 175-194.

Minas, M., Minas, A., Gourgulianis, K., & Stournara, A. J. J. j. o. i. d. (2007). Epidemiological and clinical aspects of human brucellosis in Central Greece. 60(6), 362-366.

Mode, S. (2019). Assessing intracellular persister formation and antibiotics susceptibility in "Brucella abortus". University_of_Basel,

Mohamud, A. I., Mohamed, Y. A., & Mohamed, M. I. J. J. o. I. V. S. (2020). Sero-prevalence and associated risk factors of bovine brucellosis in selected districts of Benadir Region, Somalia. 4(2), 57-63.

Mohamud, A. I., Mohamed, Y. A., & Mohamed, M. I. J. J. o. I. V. S. (2020). Sero-prevalence and associated risk factors of bovine brucellosis in selected districts of Benadir Region, Somalia. 4(2), 57-63.

Moreno, E., Cloeckaert, A., & Moriyón, I. J. V. m. (2002). Brucella evolution and taxonomy. 90(1-4), 209-227.

Mousa, A. R. M., Elbag, K. M., Kogbali, M., & Marafie, A. A. J. R. o. i. d. (1988). The

nature of human brucellosis in Kuwait: study of 379 cases. 10(1), 211-217.

Mousa, A. R. M., Elbag, K. M., Kogali, M., & Marafie, A. A. J. R. o. i. d. (1988). The nature of human brucellosis in Kuwait: study of 379 cases. 10(1), 211-217.

Muñoz, P., Marín, C., Monreal, D., Gonzalez, D., Garin-Bastuji, B., Diaz, R., . . . Immunology, V. (2005). Efficacy of several serological tests and antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to *Yersinia enterocolitica* O: 9. 12(1), 141-151.

Muñoz, P., Marín, C., Monreal, D., Gonzalez, D., Garin-Bastuji, B., Diaz, R., . . . Immunology, V. (2005). Efficacy of several serological tests and antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to *Yersinia enterocolitica* O: 9. 12(1), 141-151.

Musallam, I., Abo-Shehada, M., Hegazy, Y., Holt, H., Guitian, F. J. E., & Infection. (2016). Systematic review of brucellosis in the Middle East: disease frequency in ruminants and humans and risk factors for human infection. 144(4), 671-685.

Mutua, P. K. (2017). Pastoralists' perceptions and risk of human brucellosis in kajiado county, Kenya. University of Nairobi,

N Xavier, M., A Paixao, T., B den Hartigh, A., M Tsolis, R., & L Santos, R. J. T. o. v. s. j. (2010). Pathogenesis of *Brucella* spp. 4(1).

Narladkar, B. J. V. w. (2018). Projected economic losses due to vector and vector-borne parasitic diseases in livestock of India and its significance in implementing the concept of integrated practices for vector management. 11(2), 151.

Narladkar, B. J. V. w. (2018). Projected economic losses due to vector and vector-borne parasitic diseases in livestock of India and its significance in implementing the concept of integrated practices for vector management. 11(2), 151.

Ndaki, M. (2022). A quantitative risk assessment of humans exposure to brucellosis through the consumption of raw cow milk in Arusha, Tanzania. The University of Zambia,

Ng'ang'a, C. M. (2022). Cultural Drivers of Brucellosis and Treatment Pathways for Febrile Illnesses Among Agro Pastoralists in Kilombero District, Tanzania. University of Nairobi,

- Nielson K (2002) Diagnosis of Brucellosis by serology. *Vet Microbiol* 90: 447-59.
- Njeru, J., Wareth, G., Melzer, F., Henning, K., Pletz, M., Heller, R., & Neubauer, H. J. B. p. h. (2016). Systematic review of brucellosis in Kenya: disease frequency in humans and animals and risk factors for human infection. 16, 1-15.
- Obonyo, M. O. (2018). Sero-Prevalence And Factors Associated With Brucellosis In Goats And Sheep And Assessment Of Pastoralists, Knowledge Attitude And Practices Towards Brucellosis In Garissa County.
- Obonyo, M. O. (2018). Sero-Prevalence And Factors Associated With Brucellosis In Goats And Sheep And Assessment Of Pastoralists, Knowledge Attitude And Practices Towards Brucellosis In Garissa County.
- Olsen, S., Tatum, F. J. V. M. R., & Reports. (2016). Swine brucellosis: current perspectives. 1-12.
- Owusu-Apenten, R., & Vieira, E. (2022). Microbial foodborne disease outbreaks. In Elementary food science (pp. 171-196): Springer.
- Padilla Poester, F., Nielsen, K., Ernesto Samartino, L., & Ling Yu, W. J. T. O. V. S. J. (2010). Diagnosis of brucellosis. 4(1).
- Pal, M., Gizaw, F., Fekadu, G., Alemayehu, G., & Kandi, V. J. A. J. E. (2017). Public health and economic importance of bovine Brucellosis: an overview. 5(2), 27-34.
- Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., & Tsianos, E. V. J. T. L. i. d. (2006). The new global map of human brucellosis. 6(2), 91-99.
- Pavlik, I., Falkinham III, J., Falkinham III, J. O., Pavlik, I., Falkinham III, J., Pavlik, I., . . . Klimes, J. (2009). The occurrence of pathogenic and potentially pathogenic mycobacteria in animals and the role of the environment in the spread of infection. In The ecology of mycobacteria: Impact on animal's and human's health (pp. 199-281): Springer.
- Pelerito, A., Nunes, A., Grilo, T., Isidro, J., Silva, C., Ferreira, A. C., . . . Gomes, J. P. J. F. i. m. (2021). Genetic characterization of *Brucella* spp.: whole genome sequencing-based approach for the determination of multiple locus variable number tandem repeat profiles. 12, 740068.

Pérez-Sancho, M., García-Seco, T., Domínguez, L., & Álvarez, J. J. U. o. b. (2015). Control of animal brucellosis, The most effective tool to prevent human brucellosis. 10(61222).

PLAN, A. V. E. (2005). Disease Strategy Bovine brucellosis.

Rahman, M. T., Sobur, M. A., Islam, M. S., Ievy, S., Hossain, M. J., El Zowalaty, M. E., . . . Ashour, H. M. J. M. (2020). Zoonotic diseases: etiology, impact, and control. 8(9), 1405.

Rahman, M., Faruk, M., Her, M., Kim, J., Kang, S., & Jung, S. (2011). Prevalence of brucellosis in ruminants in Bangladesh.

Rahman, M., Faruk, M., Her, M., Kim, J., Kang, S., & Jung, S. (2011). Prevalence of brucellosis in ruminants in Bangladesh.

Refaï, M. J. V. m. (2002). Incidence and control of brucellosis in the Near East region. 90(1-4), 81-110.

Rossetti, C. A., Arenas-Gamboa, A. M., & Maurizio, E. J. P. n. t. d. (2017). Caprine brucellosis: A historically neglected disease with significant impact on public health. 11(8), e0005692.

Rossetti, C. A., Maurizio, E., & Rossi, U. A. J. F. i. v. s. (2022). Comparative review of brucellosis in small domestic ruminants. 9, 887671.

Roy, S., LaFramboise, W. A., Nikiforov, Y. E., Nikiforova, M. N., Routbort, M. J., Pfeifer, J., . . . medicine, l. (2016). Next-generation sequencing informatics: challenges and strategies for implementation in a clinical environment. 140(9), 958-975.

Roy, S., LaFramboise, W. A., Nikiforov, Y. E., Nikiforova, M. N., Routbort, M. J., Pfeifer, J., . . . medicine, l. (2016). Next-generation sequencing informatics: challenges and strategies for implementation in a clinical environment. 140(9), 958-975.

Sadhu, D. B., Panchasara, H., Chauhan, H., Sutariya, D., Parmar, V., & Prajapati, H. J. V. w. (2015). Seroprevalence and comparison of different serological tests for brucellosis detection in small ruminants. 8(5), 561.

Saxena, N., Singh, B. B., & Saxena, H. M. J. I. J. C. M. A. S. (2018). Brucellosis in sheep and goats and its serodiagnosis and epidemiology. 7(1), 1848-1877.

- Seleem, M. N., Boyle, S. M., & Sriranganathan, N. J. V. m. (2010). Brucellosis: a re-emerging zoonosis. 140(3-4), 392-398.
- Sharifi, H., Tabatabaei, S., Rashidi, H., Kazeminia, S., Sabbagh, F., Khajooei, P., . . . Leontides, L. J. I. j. o. v. r. (2014). A cross-sectional study of the seroprevalence and flock-level factors associated with ovine and caprine brucellosis in southeastern Iran. 15(4), 370.
- Sharifi, H., Tabatabaei, S., Rashidi, H., Kazeminia, S., Sabbagh, F., Khajooei, P., . . . Leontides, L. J. I. j. o. v. r. (2014). A cross-sectional study of the seroprevalence and flock-level factors associated with ovine and caprine brucellosis in southeastern Iran. 15(4), 370.
- Sharma, V., & Ganguly, S. J. I. J. o. L. R. (2017). Brucellosis, a prominent bacterial zoonosis and strategies for prevention and control: A Review. 7(8), 18-29.
- Sheik-Mohamed, A., Velema, J. P. J. T. m., & health, i. (1999). Where health care has no access: the nomadic populations of sub-Saharan Africa. 4(10), 695-707.
- Shome, R., Nagalingam, M., Priya, R., Sahay, S., Kalleshamurthy, T., Sharma, A., . . . Shome, B. J. V. W. (2020). Perceptions and preparedness of veterinarians to combat brucellosis through Brucellosis Control Programme in India. 13(2), 222.
- Sibhat, B., Tessema, T. S., Nile, E., Asmare, K. J. T., & diseases, e. (2022). Brucellosis in Ethiopia: A comprehensive review of literature from the year 2000–2020 and the way forward. 69(5), e1231-e1252.
- Tadeg, W. M., Gudeta, F. R., Mekonen, T. Y., Asfaw, Y. T., Birru, A. L., Reda, A. A. J. J. o. V. M., & Health, A. (2015). Seroprevalence of small ruminant brucellosis and its effect on reproduction at Tellalak District of Afar region, Ethiopia. 7(4), 111-116.
- Tadeg, W. M., Gudeta, F. R., Mekonen, T. Y., Asfaw, Y. T., Birru, A. L., Reda, A. A. J. J. o. V. M., & Health, A. (2015). Seroprevalence of small ruminant brucellosis and its effect on reproduction at Tellalak District of Afar region, Ethiopia. 7(4), 111-116.
- TASEW, M. S., Motbynor, A., & Tsegaye, D. (2023). SMALL RUMINANT BRUCELLOSIS: SERO-PREVALENCE, ASSOCIATED RISK FACTORS, ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICES OF

COMMUNITIES AND ECONOMIC IMPACT IN BURKA DINTU AND CHIRO DISTRICTS OF WEST HARARGHE ZONE, EASTERN ETHIOPIA. Haramaya University,

Tegegn, A. H., Feleke, A., Adugna, W., & Melaku, S. K. J. J. V. S. T. (2016). Small ruminant brucellosis and public health awareness in two districts of Afar Region, Ethiopia. 7(335), 2.

Teles, J., dos Santos, A., Silva, S., Cruz, M., & da Silva-Júnior, F. J. R. L.-a. d. E. (2015). Prevalence of *Brucella* spp in humans. 23(5), 919-926.

Virhia, J. (2020). Healthy animals, healthy people: lived experiences of zoonotic febrile illness in northern Tanzania. University of Glasgow,

Wanyoike, F., Rich, K. M., Mtmet, N., Bahta, S., & Godiah, L. J. S. R. R. (2023). An assessment of small ruminant production, marketing, and investment options in Somaliland: A system dynamics approach. 218, 106882.

Wei, X., Lin, W., & Hennessy, D. A. J. F. P. (2015). Biosecurity and disease management in China's animal agriculture sector. 54, 52-64.

Wernery, U. J. R. s. t. (2014). Camelid brucellosis: a review. 33(3), 839-857.