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Review Article

Review on Small Ruminant Brucellosis in Ethiopia

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Abstract

Ethiopia, one of the sub-Saharan African countries, is characterized by the presence of strong correlation between a high burden of zoonotic diseases and poverty. The country has the largest and second largest livestock and human population respectively in Africa. These populations have very high close contact and/or relationship between them which make the country vulnerable to the spread of different zoonotic diseases. Because of this, there are a large number of zoonotic diseases that occur endemically. Brucellosis is one of the top five neglected zoonotic diseases and has been causing significant public and economic impact both in humans and animals. Studies, conducted so far, indicate that the disease is endemic and the main challenging one. According to the studies, human brucellosis has been reported from different geographical areas of the country with sero- prevalence range between 2.15 % to 48.3% (2006 -2021). In most of studies, it becomes increase from time to time and/or its single digit before 2009 (4.8% in 2006, 3.78 in 2007 & 3.6% in 2009) to its double digit prevalence (29.4% & 34.1% in 2009;16% in 2012; 10.6% in 201; 31.5%, 34.9% & 48.3% in 2021). This increment clearly shows that how much the disease is a future threatens zoonotic disease in the country. On the other hand, small ruminant brucellosis reports (between the years 2015-2021) indicate the prevalence range of 0.24 to 13.7%. A study from Bale Zone, Oromia region reported an overall 6.5% and 2.9% animal level and 50% and 22% flock level seroprevalence tested by RBPT and CFT respectively. This untouched area report reveals how much the disease has widespread characteristics in the country. But, there is a lack of controlling activity and community awareness and information communication. Therefore, actual implementation of prevention and control measures, community awareness, further studies and continuous review to provide compiled information and to understand the transmission dynamics of the disease is required.

ABBREVIATIONS

AAU: Addis Ababa University, BC: Before Christ, BCV: *Brucella*-containing vacuole, CFT: Complement Fixation Test, DCs: Dendritic cells, ELISA: Enzyme Linked Immuno Sorbent Assay, GA: Gashaw Adane, GM: Gezahegne Mamo, LPS: Lipopolysaccharide, MRT: Milk Ring Test, NBTWG: National Brucellosis Technical Working Group, PCR: Polymerase Chain Reaction, RBPT: Rose Bengal Plate Test, RER: Rough Endoplasmic Reticulum, RES: Reticulo-endothelial systems, SNNPR: Southern Nations Nationalities People Regions, Spp.: Species, UK: United Kingdom, VPH: Veterinary Public Health, WAHIS: World Animal Health Information System

INTRODUCTION

Ethiopia, one of the sub-Saharan African countries, is characterized by the presence of strong correlation between a high burden of zoonotic diseases and poverty because of high risk of zoonotic disease transmission and emerging and reemerging pandemic threats [1]. The country has a total of about 95.37 million small ruminants. Of which, goats are constitute 42.91 million while 52.46 million are sheep in which their 76% and 75% are kept in the lowlands and highlands respectively [2]. About 80% of Ethiopians are dependent on agriculture and have a very high close relationship with their livestock. As a result, the country is vulnerable to the spread of different zoonotic diseases [3,4]. There are large number of zoonotic diseases that occurs endemically which threats the huge amount of livestock in the country [3].

Brucellosis, one of the top five zoonoses and neglected diseases in Ethiopia, has a high public and economic significant impact on the population. It causes serious economic losses in small ruminants of the country through breeding inefficiency, loss of lambs and kids, reduced wool, meat and milk production and barrier for international trade of live animals and their products [5]. In addition to supporting the livelihood of the people, particularly pastoral communities (being the source of money and food), small ruminants are the main export commodities and contribute considerable share to the country's economy. The meat of Ethiopia's small ruminant has a continuous export market to Middle Eastern, Gulf States and other African countries. But, the production is challenging by different constraints like shortage of feed and water, poor husbandry management, diseases like brucellosis and others that are resulted in the limitation the export market [6].

Brucellosis is one of the most serious infectious diseases recognized as major constraints for sheep and goats productivity and public health in Ethiopia [7]. Particularly, the habit of the people living in close proximity to their livestock, tradition of raw animal product consumption, regular contact between they and their animals, improper handling of cases and/or products by occupational workers and its widespread prevalence make the disease not only endemic but also listed among the top public health threatening zoonosis in the country [8].

Even though the so far conducted studies indicate that brucellosis is endemic in Ethiopia, awareness creation and information communication and/or dissemination among the whole community including decision makers are minimal. In addition to this, little or no studies are conducted on higher risk groups (abattoirs and laboratory institutions and their stuff), most pastoral areas that are far away from the country's capital. Therefore, knowledge of the current status of brucellosis with particular emphasis on small ruminant brucellosis and shining out the public health and economic burden of the disease is necessary. The best way to find this current information is by reviewing the previous studies which will provide compiled information for professionals, stakeholders, officials, partners and communities as a whole. Therefore, the objectives of this article are: to show the prevalence and epidemiological characteristics of brucellosis in Ethiopia, to highlight the economic and public health significance of small ruminant brucellosis and to review the status of Small Ruminant and Human Brucellosis in Ethiopia.

LITERATURE REVIEW

Background of Brucellosis

Brucellosis is an ancient disease that can be traced back to the 5th plague of Egypt around 1600 BC [9]. But, the knowledge of the disease was clearly started eighteen centuries later when Sir David Bruce isolated *Micrococcus melitensis* (now *B.melitensis*) from the spleen of a British soldier who died from Malta fever (a febrile illness) among military personnel stationed at Malta. The zoonotic nature of the brucellosis was demonstrated in 1905 following the isolation of *B. melitensis* from goat's milk which was used for soft cheese production in Malta [10]. In 1950s, over 200 cases of brucellosis were caused by ingestion of a special cheese from Maltese goats. In the 1895, 1914, and 1966, *Brucella* species were isolated from aborted bovine, swine, and canine fetuses, respectively. In 1953, *B.ovis* was identified as a cause of epididymitis in rams. In the last 15 years, 3 new non-classical species of *Brucella* has also been identified [11-13].

Brucellosis is a neglected zoonotic disease of humans and animals in sub-Saharan African countries including Ethiopia [14,15]. It is a highly contagious and considered (by World Health Organization, United States Food and Agriculture Organization and Office of International Epizootics) as one of the most serious zoonoses with considerable public health importance in the world [16]. According to OIE, brucellosis is the second most dangerous zoonotic disease in the world next to rabies. It is known by several names in which these synonyms are associated with its causative agent researchers and clinical symptoms. For example, it is called Malta fever, Undulant Fever, Mediterranean fever, Rock Fever of Gibraltar, Gastric Fever and Typho-malarial fever in human while Bang's disease, enzootic abortion, epizootic abortion, contagious abortion, slinking of calf and ram epididymitis in animals [17,18].

Etiology of the Brucellosis

Brucellosis is caused by a gram-negative, facultative intracellular bacterium from the genus **Brucella** which infects a wide range of animal species and humans. *Brucella* species are not host specific but are known to have a host preference [19], and they are classically grouped into different species based on their phenotypic and antigenic differences and host specificity. These are *B.abortus* (cattle, buffalo, elk, yaks, and camels, biovars 1–6, and 9), *B.melitensis* (goats, sheep, biovars 1–3), *B.suis* (pigs, reindeer and hares, biovars 1–5), *B.ovis* (sheep), *B.canis* (dogs) and *B.neotomae* (desert wood rats). *B. ceti* (dolphins), *B. pinnipedialis* (seals), *B. microti* (voles) and *B. inopinata* (reservoir undetermined) are also new members included recently [20,12]. *B. inopinata*, the only species not isolated from animal reservoir, was also isolated from a breast implant infection in a woman [21].

The reason for the division of *Brucella* into different species is their difference in biochemical capabilities, susceptibilities to dyes and host preference. Even though *Brucella* spp. have an affiliation to specific natural hosts, they can affect heterogeneous hosts [22,23]. Of the six classical species of *Brucella*, those which pathogenic to man are *Brucella melitensis* (the most common cause of human brucellosis), *B. abortus* (the second), *B.suis* and *B.canis* [10,12].

Epidemiology of Brucellosis

Brucellosis has been controlled and/or eradicated in most developed countries due to the fact that they have conducted extensive control programs. However, it remains an important economic and human- animal health challenge in developing countries; where large people rely mainly on their livestock. This is mainly because of resources lacking and coordinated prevention and control programs are not started. It is still a major disease problem in the Mediterranean region, western Asia, parts of Africa and Latin America [24]. There is a problem of under reporting and misdiagnosis due to the resemblance with other diseases which resulted in lagging of implementing control program [25].

Epidemiology of Human Brucellosis: Brucellosis is a disease of animals in which man is infected as terminal host and this shows that the incidence of brucellosis in man is clearly correlated to the incidence in animals [26]. Zoonotic brucellosis is the most common in the world (Figure 1), that accounting more than 500,000 reported cases annually in which high prevalence is found in African countries, including Ethiopia, due to many countries have not yet started control or eradication programs [27]. The survival of the disease in populations depends widely on host range and resistance of *Brucella* to environment as well as host immune system [28]. The prevalence of human brucellosis is also varying with standards of personal and environmental hygiene, animal husbandry practices, traditional habits of raw animal product consumption and species of the causative agent [29] (Figure 1).

Epidemiology of Animal Brucellosis: The epidemiology of brucellosis in animals is also complex and varies among different agro-ecological areas. The disease has also high incidence and prevalence in animal in Africa, Mediterranean, Middle East, and Arabian Peninsula, the Indian subcontinent, in parts of Mexico and Central and South America (Figure 2), may be due to they have not started control or eradication schemes [31] (Figure 2).

Risk Factors of Brucellosis

The initiation, spread, maintenance and/ or control of brucellosis are related to risk factors like animal population, management, biology of the disease and others [33].

Host Related Factors: All animals are comparatively susceptible and so far no specific breed is resistant to brucellosis. Age, sex and reproductive status are the main host factors and *Brucella* infection occurs in all ages with most commonly persisting in sexually mature animals, which means that sexually mature and pregnant animal are more susceptible to infection than sexually immature one [33]. Exposure to strains of the organism occurs primarily at the time of parturition of infected cows. Sero-prevalence of brucellosis increased with age and sexual maturity, but reported being low in young stock than the adults [34]. The low prevalence in young animals is may be due to the animal may harbor the organism without any detectable antibodies until their first parturition or abortion. The organism localizes itself in the regional lymph nodes and persist there without provoking antibody production until the animal is conceived and start secreting erythritol, which stimulates and supports the growth of *Brucella* organisms [35]. The reason for this is that sex hormones and meso-erythritol in male testicles and seminal vesicles and erythritol in female allantois fluid stimulate the growth and multiplication of *Brucella* organisms and tend to increase the concentration with age and sexual maturity [36]. Once aborted, infected cows usually develops immunity but remains carrier, and excrete huge numbers of *Brucella* in the fetal fluids [37].

Herd Management Factors: The unregulated movement of animals from brucellosis infected herds to free ones is the major means for the spread of the disease. Replacement or purchasing from an infected source is also potential for disease introduction to disease free herd. Improper management of reproductive tract excretion and abortion materials is the main source of infection. In lactating cows, if managed carelessly, the milk including colostrum is an important source of infection, and bacteria are excreted in milk throughout the lactation period. A contaminated environment or equipment used for milking or artificial insemination are further sources of infection too [38].

Agent Factor: *Brucella* is a facultative intracellular pathogen capable of multiplying and surviving within host phagocytes

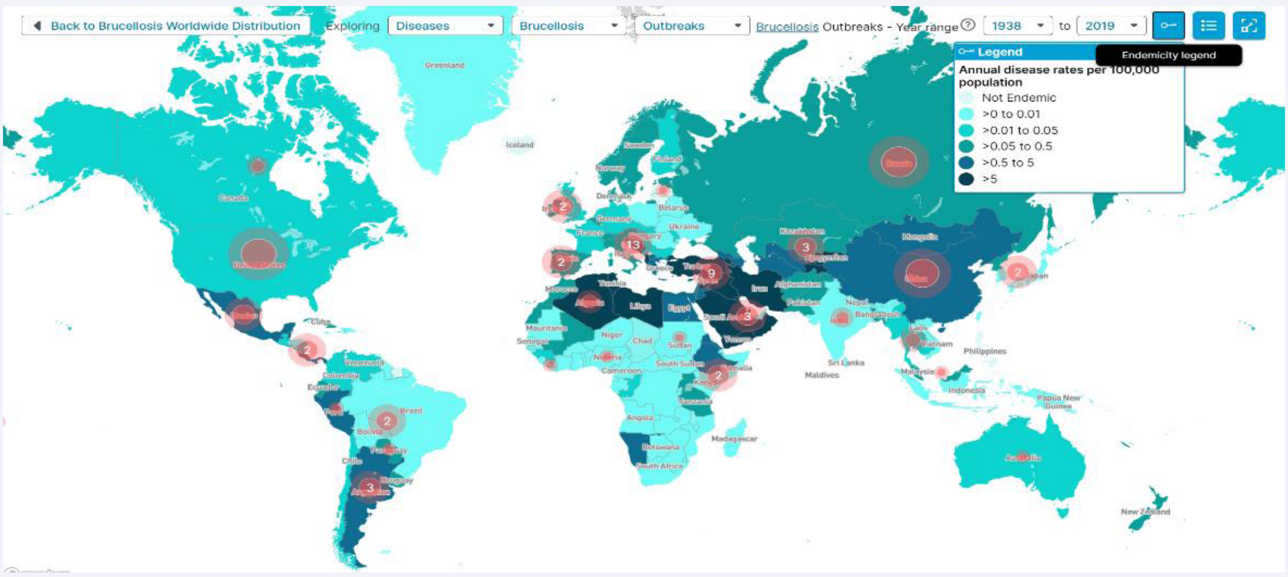

Figure 1 Brucellosis outbreaks and distribution map in the world (1938 – 2019) [30].

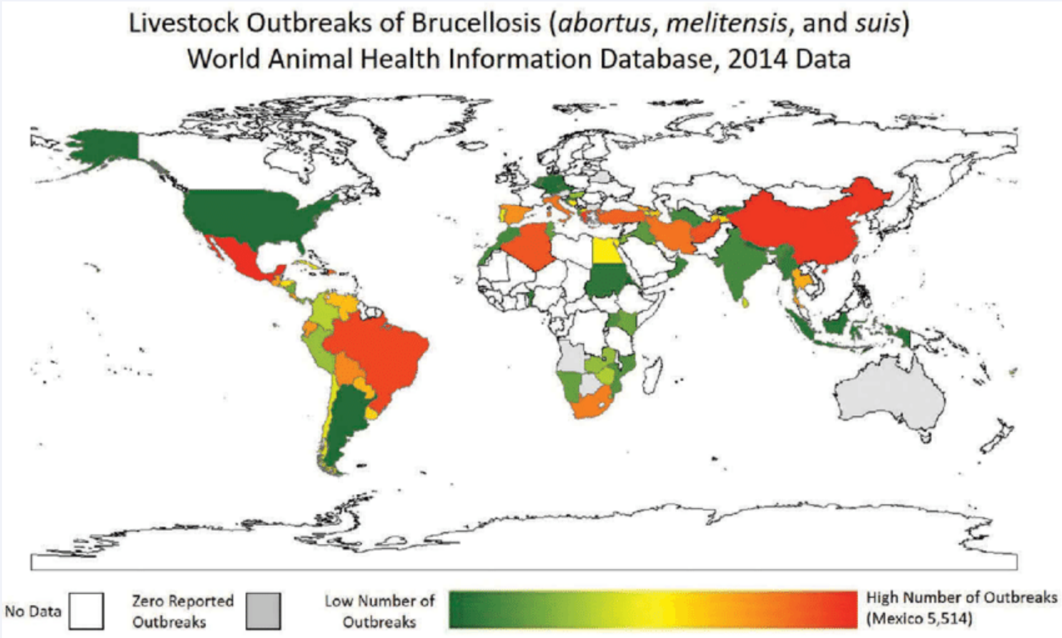

Figure 2 Heat map of Brucellosis outbreaks in livestock - WAHIS 2014 [32].

and macrophages. This is because of the organism has an outer-membrane lipopolysaccharide (LPS) and phagosome-lysosome inhibition characteristics, in which these virulent and inhibition factors are used as mechanisms for its intracellular survival and preventing itself from antibiotics. The other mechanisms used by *Brucella* are the presence of stress protein, production of catalase and superoxide dismutase which help the bacteria to resist oxidative killing [33].

Climatic and Environmental Factors: Survivability of the organism in the environment plays a great role in the epidemiology and transmission of the disease. *Brucella* may retain for several months in water, aborted fetuses, fetal membranes, feces, liquid manure, wool, hay, buildings, equipment and clothes. It is also able to withstand drying and will persist in dust and soil. Temperature, humidity and pH influence the organism's ability to survive in the environment. *Brucella* is sensitive to direct sunlight, disinfectant and pasteurization [39,40].

Human Related Factors

Age and Sex: Adults have often been commonly exposed to *Brucella* infection in chronic form. Brucellosis is considered a pediatric problem because children usually accounted for a high proportion of acute cases. In developed countries where food hygiene prevents foodborne brucellosis, the disease is highly occupational and the majority of cases are males between the ages of 20 and 45 years [33].

Cultural Habit: High prevalence reports indicates living in close proximity to livestock, habit of raw animal product consumption and contact with aborted materials are the main risk factor for human brucellosis [41]. Bacterial load in animal muscle tissues is low, but consumption of undercooked liver and spleen has been recorded in human infection [42]. Blood transfusion, organ transplantation, parenteral drug administration and sexual intercourse can be considered as risk factors for acquiring brucellosis in rare cases [39].

Occupation: Workers handling *Brucella* cultures in laboratories are at high risk of acquiring brucellosis through aerosolizing due to inadequate laboratory procedures. In addition to this, abattoir workers, farmers, veterinarians and others who work with animals and consume their products are acquiring the infection [43]. Though muscle tissue of animals contains low concentrations of *Brucella* organisms, consumption of undercooked meat can transmit brucellosis, which may be due to contamination with blood and other potential secretions. But, *Brucella* has higher concentration in liver, kidney, spleen, udder and testis and consumption of these organs and tissues undercooked has very high risk. Inhalation of contaminated dust and infected animal fluids are also the main source of infection, particularly in clinics, laboratories and abattoirs [44].

Transmission and Source of Infection

In Animals: Infection in animal is transmitted through ingestion or inhalation of organisms from different sources like aborted fetus, fetal fluids and vaginal excretions of contaminated pasture or water [38]. It is also transmitted from one herd to another by an infected animal. The disease may also be spread when wild animals from an affected herd mingle with brucellosis-free herds. Aborted and other materials are the main source of organisms for transmission to other animals and man.

Large numbers of organisms are shed at the time of parturition or abortion [45]. Transmission of horizontal is possible through ingestion of contaminated feed, skin penetration, conjunctiva, and inhalation, udder contamination during milking as well as licking of discharge from newborn or retained fetal membrane. Venereal infection is also reported and it is the primary route of transmission for *B. ovis, B.suis* and *B. canis*. *B. abortus* and *B. melitensis* can be found in semen, but the venereal transmission of these organisms is uncommon [46].

In Humans: Human becomes infected when consume unpasteurized milk and milk products, raw blood and meat (mostly liver, kidney, spleen), contact with animal materials like abortion, urine, placenta, during and in the post parturition period. The disease is an occupational that occurring most often in risk group workers [47]. Inhalation of contaminated dust or aerosols, inoculation of live vaccines (such as *B.abortus Strain 19* and *B.melitensis Rev.1*) can also cause infections [48]. Person-to-person transmission like blood donation and tissue transplantation, and from a contaminated environment are possible (Figure 3). Congenital or trans- placental infection is reported too [38] (Figure 3).

Pathogenesis of Brucellosis

The predilection site for *Brucellae* is mainly macrophages, dendritic cells (DCs) and trophoblasts. *Brucella* species can invade epithelial cells of the host and use M cells in the intestine as a portal of entry and then sequestered with in monocytes and macrophages of reticulo-endothelial systems (RES), such as lymph node, liver, spleen and bone marrow [50]. Once invaded the epithelial cell through the digestive or respiratory tract, *Brucellae* are capable of surviving intracellularly within host cells due to its ability to interfere with intracellular trafficking, preventing fusion of the *Brucella*-containing vacuole (BCV) with its lysosome markers, and directing the vacuole towards a compartment having rough endoplasmic reticulum (RER). This permits for intracellular replication of the organism [51]. Finally, the organism spreads through a hematogenous route in females to reach the placenta and the fetus.

The preferential localization to the reproductive tract of the pregnant animal is due to the presence of the allantois fluid containing erythritol that stimulates the growth of *Brucella*. Erythritol (four-carbon alcohol) is considered to be one of the factors, which is high in placenta and fetal fluid around the fifth month of gestation. An initial localization within erythrophagostic trophoblasts of the placentome adjacent to the chorio-allantoic membrane results in rupture and ulceration of the membrane. Finally, the damage to placental tissue together with fetal infection and/or fetal stress will inducing maternal hormonal changes that cause abortion [52]. *Brucella* species also have a mechanism of preventing the activation of the host innate immune system. It can also induces suppression of the transcription of pro-inflammatory mediators in trophoblastic cells at early stages of infection and placental cells are targeted during infection of pregnant animals [53].

Clinical Signs of Brucellosis

In Animals: Brucellosis can be latent infection in animals for several years and clinical manifestation is related to the reproductive tract. Usually, manifestation of the disease in females is characterized by third trimester abortion, neonatal weakness, retained placenta, endo-metritis and reduced milk yield. In highly susceptible non-vaccinated pregnant animals, abortion after the 5th month of pregnancy is cardinal feature of the disease. Once aborted, an infected animal usually develops immunity but remains carrier and can give subsequent calving followed by excreting large numbers of *Brucella* in the fetal fluids. Abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility. Orchitis, epididymitis and subsequent infertility are also in males. Polyarthritis and/or hygroma in chronic case and vaginitis are some of the observed signs in animals [54]. Aborted fetuses may have excess blood-stained fluids in body cavities, with enlarged

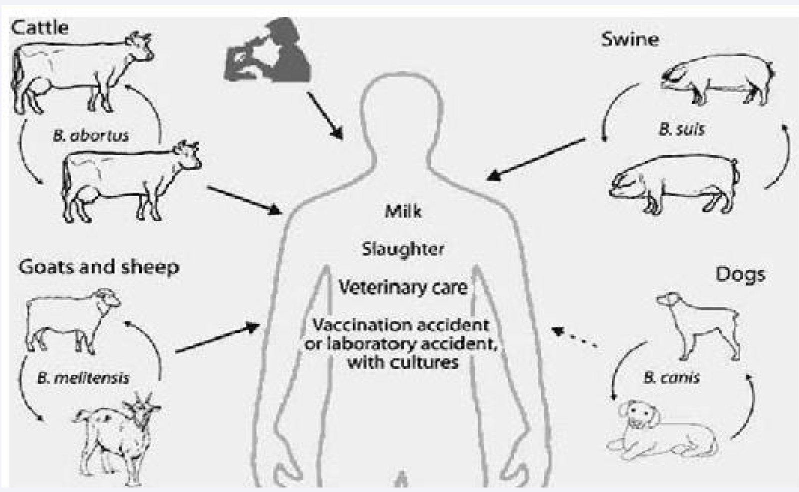

Figure 3 Transmission of Brucella to human [49]

spleen and liver. Mild interstitial inflammatory reaction in the mammary gland may also be observed, which is associated with elimination of bacteria in the milk. In the chronic stage of the disease, the epididymis can be increased in size [55].

In Humans: The incubation period of brucellosis in humans varies between 7 and 65 days. It can be confused with malaria and influenza and can progress to a chronically debilitating disease with severe complications, like in bone and joint involvement, neuro-brucellosis, endocarditis. Abortion, intermittent or irregular fever, profuse sweating, anorexia, malaise, weight loss, depression, headache, joint pains and epididymo-orchitis are common signs. Abortion may happen during the early trimesters of pregnancy. In the chronic form, it may result in serious complications in which the musculoskeletal, cardiovascular and central nervous systems are affected [38,56].

Methods of Diagnosis for Brucellosis

Bacteriological Methods: Bacteriological methods are the gold standard diagnostic method of brucellosis for isolation of the agent due to they are specific, allows bio typing, and from the epidemiological point of view [57]. *Brucella* is a fastidious bacterium and requires rich media for primary cultures. Furthermore, its isolation requires a large number of viable bacteria in clinical samples, proper storage and quick delivery to the diagnostic laboratory [58]. The samples of choice for isolation of *Brucella* are fetal membranes, particularly placental cotyledons, milk, vaginal secretions, arthritis or hygroma fluid, semen in males and fetal organs such as lungs, bronchial lymph nodes, spleen, liver and gastric contents where the number of organisms are very high in those organs and tissues. Vaginal secretions can be sampled after abortion or parturition, preferably using a swab with transporter medium, allowing isolation of the organism up to six weeks post parturition or abortion. Milk samples should be a pool from all four mammary glands and non-pasteurized dairy products can also be sampled for isolation. Mammary, iliac, pharyngeal, parotids and cervical lymph nodes and spleen are samples of choice in slaughterhouses. Samples must be immediately sent to the laboratory using frozen temperature at -20 ºC (+4 ºC for milk sample), and must be identified as suspect of *Brucella* spp. infection [59,60].

Molecular Methods: These methods are important tools for diagnosis and epidemiologic studies, providing relevant information for identification and biotypes of *Brucella* species, allowing differentiation between virulent and vaccine strains [61]. PCR techniques like Multiplex PCR typing and Real-time PCR are the most broadly used molecular technique for brucellosis diagnosis [62].

Serological Methods: Most of the time, the control of brucellosis depends on these methods due to their crucial for laboratory diagnosis. They can be classified as screening tests, monitoring and surveillance tests, complementary or confirmatory tests (complement fixation, ELISA, Fluorescence assays) [63]. In serological tests, though the presence of anti-*Brucella* antibodies suggests exposure to *Brucella* spp., it does not indicate that *Brucella* species induced production of those antibodies or does not necessarily mean that seropositive animals have current or active infection of *Brucella* at the time of sampling. Therefore, the "gold standard" diagnosis in brucellosis remains in the isolation of *Brucella spp*. If brucellosis is suspected in livestock or in wildlife because of positive serological results, isolation of the organism is mandatory [64]. The main ones include:

Rose Bengal Plate Test (RBPT): it is a rapid, slide-type agglutination assay applied with a stained *B. abortus* suspension at pH of 3.6-3.7 and plain serum. It is simple and an ideal screening test for small laboratories with limited resources. RBPT is an agglutination test that is based on reactivity of antibodies against smooth LPS. WHO recommends the confirmation of RBPT by other assays such as serum agglutination tests [65].

Enzyme Linked Immuno Sorbent Assay (ELISA): It is a test of choice for complicated cases and is standard assay for serological diagnosis of brucellosis. This method measures IgG, IgA and IgM antibodies that allows an interpretation of the clinical situation [66]. ELISA is the best for screening large populations for *Brucella* antibodies and for differentiation between acute and chronic phases of the disease [67]. Indirect ELISA is used for serologic diagnosis of brucellosis in sheep, goats and pigs as well as serum and milk from cattle. Competitive ELISA (cELISA) is used for detection of anti-*Brucella* in serum samples from cattle, sheep, goats and pigs. It can differentiate vaccine antibody response from actual infections [68].

Complement Fixation Test (CFT): This test has accuracy and is usually used as confirmatory diagnosis for *B. abortus, B. melitensis, and B. ovis* infections. It is also the reference test recommended by the OIE for international transit of animals [69].

Other Important Tests

Milk Ring Test (MRT): It is based on agglutination of antibodies secreted into the milk and allows screening of large numbers by using milk samples. The test is classified as surveillance or monitoring test as it is used for monitoring herds or areas free of brucellosis. During this test, a positive result indicates the presence of infected cattle in the herd so that the test should be followed by an individual serological test of the entire herd [70].

Brucellin Skin Test: It is an allergic test that detects the specific cellular immune response induced by *Brucella* spp. infection. It is applied by injection of brucellergene, a protein extract of strain of *Brucella* spp., followed by a local inflammatory response in a sensitized animal. It could be used as a confirmatory test on non-vaccinated animal and considered as an alternative test by the OIE [71].

Treatment of Brucellosis

In humans, the main goal of treatment in brucellosis is to control the illness and prevent complications, relapses and sequelae. The main principles of treatment are the use of combination regimens, and prolonged treatment duration. Antibiotic treatment should be implemented at as early a stage as possible [72]. Doxycycline (100mg orally twice a day for 6 weeks) with Gentamicin (5mg/kg once daily for the first 7 days) are the drugs of choice [38]. It is ineffective in animals because of the bacteria is intra cellular. But, it can be tried as control schemes and infected cattle can be tried to treat with tetracycline intravenously daily for 3-6 weeks. But, neither streptomycin nor doxycycline alone can prevent multiplication of intracellular *Brucella*. After treatment, the animal may shade the bacteria and can be source of infection [73].

Significance of Brucellosis

Economic Significance: Brucellosis is responsible for considerable economic losses in livestock by causing infertility, decreased milk production and abortion storms in herds. The primary economic impact of brucellosis is loss of production mostly characterized by 20% of a decrease in milk production. Abortion, associated with retained placenta, metritis and permanent infertility which resulted in culling of productive animals is the leading cause. Acute metritis because of retention of placenta is also the main which resulted in death. In general, economic crisis due to loss of lambs and kids following abortion, decrease milk production, cull and condemnation due to failure of breeding, restriction of export trade, medical and research cost as well as affecting of productive man power are the usual consequences of the disease [33].

Public Health Significance: Brucellosis is an occupational disease, occurring most often in veterinarians, farmers, stock inspectors, abattoir workers, laboratory personnel, butchers and hunters [74]. Human brucellosis is the most common zoonosis in the world and annually about 500,000 new cases is diagnosed worldwide. Consumption of sheep and goat milk which contain *B. melitensis* is the main source of human brucellosis and this is characterized by frequent outbreaks [9]. Human brucellosis may reduce productivity because of its prolonged illness resulting in loss of strength, loss of income emanated from lost working time, long-term treatment, medical care and other costs [75].

Bioterrorism: Due to highly infectious by aerosol, *Brucella* could be considered as a potential agent of biological terrorism, particularly *B. melitensis* which has been estimated that only 10-100 organisms are needed to constitute an infectious aerosol for humans. Cases of laboratory-acquired brucellosis are the perfect examples of airborne spreading of the disease. *Brucella* could be used to attack human and/or animal populations, intentionally. In human, it leads to spontaneous abortions and intrauterine fetal death in pregnant women. The first agent contemplated by the United States Army as a potential biologic weapon and still considered in that category is *B.suis* [44,38].

Control and Prevention of Brucellosis: The main goal of controlling the disease brucellosis in animals is to reduce the consequence of the disease to human health and the economic crisis. The most important principles of control majors for animal brucellosis are test and isolation and/or slaughter, hygiene, restriction of animal movement and vaccination [76,77]. In humans, the best way to prevent brucellosis infection is pasteurization of dairy products, cooking meat carefully, taking care while handling abortion materials and new born, wear protective clothing at risk areas and extremes care at the time of application for S19, RB51 or Rev 1 *Brucella* vaccines with providing of community awareness about these things [38]. There is no vaccine invented for human so far. Therefore, lack of vaccine for humans, its long treatment time, possible bio-terrorist ability of the agent and due to it has been affecting about a half million people annually make the disease a global health threat [78].

Status of Small Ruminant and Human Brucellosis in Ethiopia

The first case of brucellosis in Ethiopia was reported in the 1970s. Since then, several sero-prevalence of brucellosis has been reported from different parts both in human and animal. These reports indicate that animal and human brucellosis is distributed among different localities, agro-ecology and production systems [79]. The disease is still remaining as the main challenging and prioritized and/or listed as one of the top zoonotic diseases. Due to this, the government of the country has launched the National Brucellosis Technical Working Group (NBTWG) to spearhead collaborative efforts among government sectors, partners and universities to coordinate and synergize brucellosis prevention and control efforts through a One Health approach [3]. Although the disease has widespread characteristics in humans and their livestock (in Ethiopia) particularly in pastoral communities, there is limited information on the prevalence, transmission and risk factor of the disease among communities [80].

Status of Human Brucellosis in Ethiopia: Brucellosis causes major human health crises to the entire community in the economy affected and agrarian economy countries, including Ethiopia. In Ethiopia, brucellosis has a widespread prevalence and endemic characteristics due to the determination of associated risk factors, health intervention are not routinely exercised, lack of appropriate and effective diagnostic facilities, the presence of common close contact between humans and their livestock (up to sharing the same housing particularly in pastoral community and high risk groups) and the country has not yet started a coordinated prevention and control programs [81]. According to several seroprevalence studies which have been carried out from the mid-2000s up to 2021 (Table 1), human brucellosis has been reported from different geographical areas of Ethiopia with prevalence ranging from 2.15 % to 48.3% [82,80]. A recent integrated human-animal brucellosis sero-surveillance study in the pastoral Afar and Somali regions of Ethiopia recorded an ELISA based seroprevalence of 48.3 % and 34.9% in Afar and Somali regions respectively [80]. Another cross sectional seroprevalence study was carried out in Afar in the same year (2021) and showed a prevalence of 31.5% [83]. The highest prevalence was recorded in pastoral communities of Afar, Somalia, Oromia (Borena) and SNNP (Hammer) regions due to the habit of unpasteurized dairy products consumption, living close proximity with animals and poor access to health services

[80,81,83,84]. Isolation of the organism from human cases in Ethiopia is not recorded except one report indicating *B. melitensis* Biovar 1 isolated in the UK from a man who has Ethiopian origin [85] (Table 1).

Status of Small Ruminant Brucellosis in Ethiopia: Small ruminant brucellosis is endemic and widely distributed in Ethiopia, which has been causing high economic losses as well as becoming a serious public health threat [93]. The distribution or prevalence of small brucellosis prevalence in Ethiopia is varied from place to place and time to time may be due to differences in animal production and management system, community living standard and awareness level as well as agro ecological conditions of those study places (Figure 4).

Different seroprevalence study reports which have been conducted since 2015 shows the disease is endemic in Ethiopia [45]. This seropositive prevalence may be due to natural infection because of no brucellosis prevention and control vaccination history in Ethiopia [15]. Recently, a researche reported an overall animal level prevalence of 6.5% and 2.9%, and flock level prevalence of 50% and 22% tested by RBPT and CFT respectively in Bale Zone, Oromia region. Another very recent study in the pastoral Afar and Somali regions of Ethiopia was recorded an ELISA based seroprevalence of 9.7% and 8.9% for goat and sheep and 9.5% and 6.6% for goat and sheep in Afar and Somali regions respectively [80].

Table 1: Sero-prevalence of human brucellosis in Ethiopia (2006 -2021)

Location of study	Year of study	Study subjects	Type of test	Prevalence in %	Reference
Afar	2021	Pastoralists	ELISA	48.3	[80]
Somalia	2021	Pastoralists	ELISA	34.9	[80]
Afar	2021	Patient	ELISA	31.5	[83]
SNNPR(Borena)	2020	LS owners	RBPT, ELISA	2.6	[15]
Afar	2018	Patients	CFT	4.4	[86]
SNNPR	2017	Blood donors	NS	10.6	[87]
Oromia/ Adamitulu	2014	LS owners	RBPT, CFT	2.15	[82]
Afar	2012	Community	RBPT, CFT	16	[42]
Amhara(West Gojjam)	2009	Patients	RBPT	2.6	[88]
Oromia(Borena)	2009	Patients	LFA	34.1	[81]
SNNP/Hammer	2009	Patients	LFA	29.4	[81]
Oromia/Jimma	2008	Patients	RBPT, CFT	3.6	[89]
Amhara/ Bahirdar	2007	HRG	RBPT, CFT	5.3	[90]
Sidama	2007	HRG	RBPT, CFT	3.78	[91]
Addis Ababa	2006	HRG	RBPT, CFT	4.8	[92]

HRG (High Risk Group), LS (Livestock), LFA (Lateral Flow Assay)

A cross sectional seroprevalence study in Yabello and Dire districts of Borena Zone of Oromia, Ethiopia, reported an overall prevalence of 8.8% small ruminant brucellosis tested by RBPT and ELISA [94]. 21% of seroprevalence among small ruminants with a history of abortion was also reported from SNNPR of South Omo [95]. A CFT based seroprevalence studies which conducted between 2018 and 2019 were also reported an overall small ruminant brucellosis prevalence of 0.27% in Harer (Babile); 4.5% and 12% in SNNPR (Nechisar National Park); 2.6% in Dire-Dawa and 4.98% in South Wolo and North Shewa Zones of Amhara region [96-99]. In 2017, several small ruminant brucellosis seroprevalence studies were conducted by different scholars in Oromia, Tigray, Afar, SNNPR and Somalia and showed a prevalence range 1.37% to 12.4%. Between the years 2015 and 2016, 0.7 % (Amhara), 1.99 % (Debre-Zeit and Modjo Export Abattoirs), 4.3 % & 12% (SNNPR-Gamogofa) and 13.7 % (Afar) RBPT tested seroprevalence results were recorded (Table 2). According to this review data, small ruminant brucellosis is still endemic in the country and at its increasing level as humans brucellosis does. A study with an overall animal level prevalence of 6.5% and 2.9% and flock level prevalence of 50% and 22% tested by RBPT and CFT respectively in untouched areas of Oromia region indicates how the disease has wider spread prevalence in the country [100] (Table 3).

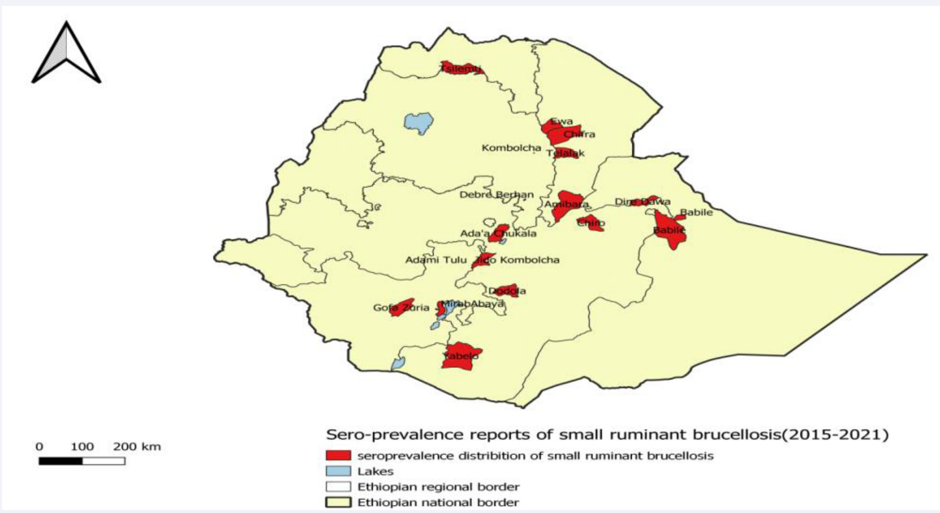

Figure 4 Distribution of small ruminant brucellosis Sero prevalence (2015-2021), developed by the authors.

Table 2: Small Ruminant Brucellosis Sero-prevalence Reports in Ethiopia (2015-2021)

Study Area		Year of publication	Number of sample	Type of Test	Prevalence in % (95%,CI)			Reference
Region	Specific Location				RBPT	CFT/ ELISA	95%,CI	
Afar	(in 7Districts)	2021	2099	ELISA		9.7,8.9	8.5–11.4	[80]
Somalis	(in 6 Districts)	2021	1243	ELISA		9.5,6.6	6.7–11.9	[80]
Oromia	Bale	2021	384	RBPT, CFT	6.5,50	2.9, 22	2.26-46.8	[100]
Oromia	Chiro	2021	2070	RBPT, CFT		0.24	1.33-3.36	[6]
SNNPR	S/Omo	2020	124	RBPT, CFT		21	0.14 -0.28	[95]
SNNPR	Borena	2020	882	RBPT,ELISA		3.2	2.1-4.6	[15]
Oromia	Borena	2020	314	RBPT,ELISA	NS	8.8	5.4-13.2	[94]
Harare	Babile	2019	384	CFT	NS	0.78	1.56	[96]
SNNPR	Nechisar	2018	246, 46	CFT	NS	4.5, 12.8	4.8–25.7	[97]
D/Dawa	D/Dawa	2018	424	CFT	NS	2.6	4-31.2	[98]
Amhara	S/W &N/S	2018	2409	NS	4.98	4.89	3.24-6.9	[99]
Oromia	Borena/Yabelo	2017	283	ELISA	NS	8.1	5.2, 11.9	[101]
Tigray	Tselemt	2017	558	CFT	NS	1.79	2.18	[102]
Oromia	Adamitulu	2017	840	CFT	NS	4.5	5.12	[103]
Afar	Amibara	2017	226	RBPT,CFT	12.4	7.52	7.41	[104]
SNNPR	Meirab-Abaya	2017	389	CFT	5.1		6.8	[105]
Somali	Jigiga/Gursm	2017	291	CFT	1.37		0.22-3.22)	[55]
SNNP	Gamo Gofa	2016	1000	RBPT	4.3	3.7	1.9%	[4]
Afar	Chifera, Ewa	2016	1190	RBPT	12.35		1.38	[8]
Afar	Tellalake	2015	414	RBPT	13.7		14.4	[106]
Oromia	D.Z & M. EA	2015	853	RBPT,CFT	1.99	1.76	0.88-2.64	[93]
Amhara	Kombolcha	2015	714	RBPT,CFT		0.7	2.1	[7]

D/Dawa (Dire-Dawa), D.Z & M.EA (Debre -Zeit and Mojo Export Abattoirs), S/W &N/S (South Wolo and North Shewa), S/Omo (South Omo)

Table 3: Isolated Brucella Species from Small ruminant in Ethiopia

Location/Source of Sample	Year of Isolation	Year of publication	Animal spp.	Method of Isolation	Isolated Brucella spp.	Reference
Afar(Amibara)	2016	2019	Goat	CFT, ELISA & PCR	*B.melitensis*	[108]
Pastoral and agro-pastoral lowlands	2007	2015	Goat	RBPT,CFT, & PCR	*B.melitensis*	[107]

Isolation of *Brucella* Organism from Small Ruminant: So far, only two isolations (*Brucella melitensis* spp.) were achieved from small ruminants in Ethiopia (Table 3). The first isolation of *Brucella melitensis* species was tried in 2015 by a researcher and his colleagues [107]. 285 goat samples were collected from three export abattoirs and tested by RBPT and CFT. Then, tissue samples were collected from 14 strongly positive goats and cultured in dextrose agar and *Brucella* agar base. Finally, *Brucella melitensis* was isolated from 2 of the 14 analyzed samples using staining, biochemical tests and polymerase chain reaction technique [107]. The second *Brucella* spp. that isolated using bacteriological and molecular methods was from Afar region, north-eastern Ethiopian 2016. Based on 28 milk and 27 vaginal swab samples collected from goats with history of abortion and reproductive problem (after serologically tested by RBP, CFT and ELISA techniques), eight *Brucella melitensis* spp. were isolated using PCR technique. Of which, three isolates were from milk samples while five were from vaginal swabs [108].

CONCLUSIONS

Brucellosis, the second most dangerous zoonotic in the world next to rabies, is considered by the World Health Organization, United States Food and Agriculture Organization and Office of International Epizootics as one of the most serious diseases. The disease is neglected and has high public and economic significance in Ethiopia. Several small ruminant brucellosis sero-prevalence and little *Brucella* isolation reports have already been recorded from different areas of the country shows that the disease is endemic and still remaining as the main challenging zoonotic diseases. These reports indicate the disease is characterized by high economic crisis and significant public health risk to the entire community, particularly in high risk groups and pastoralists. This is may be because of the community's traditional habit for raw animal products consumption, close contact with livestock and livestock products; lack of access to health service and due to the county not yet started the prevention and control measures. The economic losses of the disease is mostly sever in animal production and/or productivity, particularly in small ruminants through exerting breeding inefficiency, loss of lambs and kids, reduced wool, meat and milk production and barrier for international trade as they live and their products. In most studies, the prevalence of the disease has become increased from time to time and/or from its single digit to its double digit rate. The country has prioritized and listed the disease as one of the top five zoonotic diseases followed by the established National Brucellosis Technical Working Group in order to coordinate the prevention and control efforts through a One Health approach. However, information communication and dissemination, community awareness and practical implementation of national prevention and control measures are lagging. In addition to this, little or no studies are conducted particularly on higher risk groups and most pastoral areas that are far away from the country's capital.

Based on the conclusion, hereunder recommendations are forwarded.

- ✓ Further studies should be conducted particularly on higher risk and untouched areas.
- ✓ Actual implementation of prevention and control measures with community awareness should be started.
- ✓ Continuous review to compile and provide current information and to understand the transmission dynamics of the disease is mandatory.

DECLARATION

Authors Contributions

GA collected all the required data, designed the review and drafted the manuscript and GM advising in the designing the review and continuously supporting during the review period. All authors have read and approved the manuscript.

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