World Journal of Veterinary Science

ISSN: 2689-5951

Review Article

Epidemiology, Economic and Public Health Importance of Small Ruminant *Brucella* Infection in Ethiopia

Dereje Tulu Robi*

Ethiopian Institute of Agricultural Research, Tepi Agricultural Research Center, Ethiopia

Abstract

Brucellosis is a serious economic and public well-being concern throughout the world. It is a global spreading and causes a serious problem to developing country similarly to Ethiopia. *B. melitensis, B. ovis*is the foremost reason for this disease in the small ruminant. Moreover, *B. abortus*, the reason for brucellosis in small ruminants occasionally. It is recognized by causing abortion in the third trimester, retained fetal membrane and sterility in female, and orchitis and bursitis in the bull. *Brucella* organism is usually transmitted to different small ruminant through direct or indirect contact with diseased animals or their discharge. Humans procure the disease mostly by drinking unpasteurized milk/products and contact with diseased cattle or their discharges. The incidence of the disease is influenced by a variety of factors associated with the management system, host and environmental factors. Age, sex, species, flock size, hygienic status, and agroecology are among the most important factors of the disease. Work-related contact is observed in peoples who interaction with diseased cattle and their tissues. The most appropriate method of *Brucella* infection control is the vaccination of young female cattle. *B. melintesis* may be eradicated by isolation of diseased animals, giving the vaccine and test-slaughter methods. Thus, it is important to conduct applicable control methods, increase public awareness on the zoonotic transmission of brucellosis and conducted a study on the epidemiology of brucellosis in the higher risk group.

Keywords: Epidemiology; Economic; Zoonosis; Small ruminant; Ethiopia

Introduction

Brucella infection is one of the major bacterial agents that cause tremendous economic losses due to abortion in sheep and goat flocks in many countries [1]. *Brucella* organism is a small, gram-negative, intracellular parasite causing chronic disease which usually persists for life. Brucellosis in small ruminants is mainly caused by *B. melitensis* and *B. ovis* and in sporadic cases *B. abortus*, and *B. melitensis* are infected sheep and goats [2,3]. The disease is manifested by late-term abortions, weak lambs and kids, stillbirths, infertility and characterized mainly by placentitis in pregnant female animals, epididymitis and orchitis in males, with excretion of the organisms in uterine discharges and milk in female animals [4]. It also causes a considerable loss of productivity through high morbidity [2]. Sheep and goats brucellosis infection is widespread worldwide. However, the disease is a serious problem in developing countries [5].

Several animal species are affected with brucellosis including humans, especially those produce food animals (sheep, goats and other species) [6,7]. In sexually mature sheep and goats, brucellosis restricts to the reproductive tract and typically causes placentitis and abortion in pregnancy one. Hence, the disease causes significant losses in the reproduction and productivity of sheep and goats through high morbidity. *B. melitensis* is considered to have the highest zoonotic potential followed by *B. abortus* and *B. suis*. The disease is also categorized as one of the neglected zoonoses with serious veterinary and public health important throughout the world [8,9]. *B. melitensis* and *B. abortus* are zoonotic pathogens which cause brucellosis in human [2,10].

The economic and public health impact of *Brucella* infection remains the problem in developing countries [4]. Brucellosis results in an obstacle to trade of animals, animal products and animal movement. It causes economic losses because of abortion or breeding failure in the affected animal population, diminished milk production and inhuman brucellosis result in reduced work capacity through the sickness of the affected people [5].

The occurrence of brucellosis is influenced by several risk factors related to production systems, the biology of the individual host and environmental factors. These include age, herd size and composition, the hygienic status of the farm, the rate of contact between infected and susceptible animals, farm biosecurity and climate [2,11]. The presence of small ruminant brucellosis in Ethiopia was reported [12].

Epidemiological, economic and zoonosisinformationis therefore required to provide evidence on the importance of brucellosis in sheep and goats production in Ethiopia. The evidence can inform policymakers and interventions aimed at reducing the impacts of the disease. Hence, the present paper was designed to review the epidemiology, economic and public health importance of brucellosis in small ruminants in Ethiopia.

Brucellosis in Small Ruminant

Brief historical overview of brucellosis

The history of brucellosis does not begin with the isolation and identification of *Brucella* organisms in the 1880's. Many historical

Citation: Robi DT. Epidemiology, Economic and Public Health Importance of Small Ruminant Brucella Infection in Ethiopia. World J Vet Sci. 2020; 2(1): 1007.

Publisher Name: Medtext Publications LLC

Manuscript compiled: Jan 08th, 2020

***Corresponding author:** Dereje Tulu Robi, Ethiopia Institute of Agricultural Research, Tepi Agricultural Research Center, P.O. Box 34, Tepi, Ethiopia, E-mail: derejetulu5@gmail.com

accounts of diseases before this time could be describing brucellosis including abortion epidemics in animals and fever in humans. Other than references in animal abortions, one of the earliest recorded descriptions of brucellosis was made by Marston in 1859 [13]. The pathological evidence from the partial skeleton of the late Pliocene *Australopithecus africanus* suggests that brucellosis occasionally affected our direct ancestors 2.3-2.5 million years ago. However, brucellosis is named after Sir David Bruce, who in 1886 isolated the causative agent from a soldier in Malta where the disease caused considerable morbidity and mortality among British military personnel. During the 19th century, brucellosis was known as Malta or Mediterranean fever. In 1897, Danish veterinarian Bernhard Bang isolated *B. abortus* as the agent; and the additional name "Bang's disease" was assigned. The popular name "undulantfever" originates from the characteristic undulance (or "wave-like" nature) of the fever, which rises and falls over weeks in untreated patients. Also, different synonyms of brucellosis include Malta fever, Mediterranean fever, Gibraltar fever, Cyprus fever, Rock fever enzootic abortion, Epizootic abortion, and contagious abortion in animal and human [10].

Etiology

Brucella species are small, gram-negative, non-motile, non-spore forming bacteria function as facultative intracellular parasites, causing chronic disease which usually persists for life [2,3]. Brucellosis in small ruminants is mainly caused by *B. melitensis* and *B. ovis* and in sporadic cases *B.abortus*, and *B. melitensis* are most commonly infects sheep and goats. Breed susceptibility is variable in sheep, but goat breeds are highly susceptible. *B. ovis* primarily affects sheep [14]. Sheep and goats *Brucellosis* infection is widespread worldwide. But the disease is a serious problem in developing countries [5].

Clinical manifestation

Brucellosis suspected in any flock with a history of abortion during the last stage of pregnancy, infertility, orchitis, epididymitis, stillbirths and neonatal mortality [15]. The major clinical sign in the first stage of the disease is abortion, but other signs due to localization of the organism may be observed. These signs include orchitis, epididymitis, hygroma, arthritis, metritis, and subclinical mastitis among others [2]. However, numerous animals develop a self-limiting infection or they may become asymptomatic latent carriers and potential excretors [2]. Infection is not established if the female is exposed to the organism at the end of the pregnancy. The second stage is characterized by either elimination of *Brucella* or more frequently, by persistent inflammation of mammary gland and supramammary and genital lymph nodes, with constant or intermittent shedding of the organisms in milk and genital secretions. Animals generally abort once during the mid-third of gestation but re-invasion of the uterus occurs in subsequent pregnancies with shedding in fluids and fetal membranes. The pregnancy can also get to full- term [15]. Females that are born into an infected area and get infected generally abort less than others. This explains the high level of abortions in newly infected herds and their relatively low frequency in herds where the infection is enzootic. The udder is a very important predilection site for *Brucella* organisms. Infection in lactating, none pregnant goats is likely to lead to colonization of the udder with excretion of *Brucella* organisms in the milk [2]. Retention of placenta and metritis are common sequels to abortion. Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility [16].

Pathogenesis

B. melitensis can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, the gastrointestinal tract, and reproductive tracts. In the alimentary tract, the epithelium covering the ileal Peyer's patches is the preferred site for entry [17]. Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them and are also phagocytosed by macrophages. In macrophages, *B. melitensis* inhibits the fusion of phagosome and lysosome and replicates within compartments that contain components of the endoplasmic reticulum [18]. In ruminants, *Brucella* organisms bypass the most effective host defenses by targeting embryonic and trophoblastic tissue. In cells of these tissues, the bacteria grow not only in the phagosome but also in the cytoplasm and the rough endoplasmic reticulum. In the absence of effective intracellular microbicidal mechanisms, these tissues permit exuberant bacterial growth, which leads to fetal death and abortion [19].

Diagnostic techniques of brucellosis

The most reliable and unique method for diagnosing animal brucellosis is the isolation of *Brucella* species [20]. Diagnostic tests are applied for the following purposes: confirmatory diagnosis, screening or prevalence studies, certification, and, surveillance to avoid the reintroduction of brucellosis through the importation of infected animals or animal products [21]. The diagnostic methods include direct tests, involving isolation of organism or DNA detection by Polymerase Chain Reaction (PCR) based methods and indirect tests, which are applied either *in vitro* (mainly to milk or blood) or *in vivo* (allergic test). Isolation of *Brucella* species or detection of *Brucella* species DNA by PCR is the only method that allows certainty of diagnosis. A definitive diagnosis of brucellosis is based on culture, serologic techniques or both. Presumptive evidence of brucellosis is provided by the demonstration by modified acid-fast staining of organisms of *Brucella* in abortion material or vaginal discharge, especially if supported by serological tests. Whenever possible, *Brucella* species should be isolated using plain or selective media by culture from uterine discharges, aborted fetuses, udder secretions or selected tissues, such as lymph nodes and male and female reproductive organs. Species identified by using cultural, biochemical and serological criteria. Polymerase Chain Reaction (PCR) can provide a complementary method based on specific genomic sequences [9,22].

Serological diagnosis: Rose Bengal Plate Test (RBPT), Complement Fixation Test (CFT) and more recently ELISA, have been widely used for the serological diagnosis of brucellosis in sheep and goats [23]. They are also the official tests for international trade [24,25]. Serological tests cannot differentiate between *Brucella* species and cannot, therefore, identify which species has induced host antibodies. Therefore, only isolation of the species or specific DNA detection by Polymerase Chain Reaction (PCR), allows identification of the infecting strain [21,26].

Rose bengal plate test (RBPT): This test was developed for the diagnosis of bovine brucellosis to differentiate specific *Brucella* agglutinins from non-specific factors. When the antigen was buffered at pH 4.0 they observed that agglutination of *B. abortus* cells by non-specific agglutinins of bovine serum was inhibited whereas the activity of specific *Brucella* antibodies was not affected. Despite the scanty and sometimes conflicting information available [22], this test

is internationally acknowledged as the test of choice for the screening of brucellosis in cattle as well as in small ruminants [8,27].

However, the standardization conditions suitable for diagnosing cattle infection [24,27] are not adequate in sheep and goats and account for the low sensitivity of RBPT in small ruminants. If the antigen is standardized differently, to give higher analytical sensitivity, the diagnostic sensitivity to *B. melitensis* infection will be improved. The RBPT is based on the detection of specific antibodies of the IgM and IgG types but more effective in detecting antibodies of the IgG1 type than the IgG2 and IgM types. Also, the low pH (3.65) of the antigen enhances the specificity of the test by inhibiting nonspecific agglutinins. The temperature of the antigen and the ambient temperature at which the reaction takes place may influence sensitivity and specificity [23]. The RBPT could be modified for testing of sera in endemic, low prevalence areas to increase the sensitivity of the test. This simple modification is achieved by increasing slightly the number of sera for the test dose from 25 µl to 75 µl, at the same time maintaining the antigen volume at 25 µl. This results in a significant increase in the sensitivity of the test without affecting the specificity [28,29].

Complement fixation test (CFT): A complement fixation test is the most widely used confirmatory test and recommended by OIE [30]. As in cattle brucellosis, there is agreement that this test is effective for the serological diagnosis of brucellosis in sheep and goats despite the complexity and the heterogeneity of the techniques used in different countries. The CFT is based on the detection of specific antibodies of the IgM and IgG1 that fix complement. It is highly specific but laborious and requires highly trained personnel as well as suitable laboratory facilities. Its specificity is very important for the control and eradication of brucellosis but may test negative when antibodies of the IgG2 type hinder complement fixation [22,23].

Epidemiology of brucellosis

Geographical distribution: The geographical distribution of brucellosis is constantly changing, with new foci emerging or reemerging. The epidemiology of human brucellosis has drastically changed over the past few years because of various sanitary, socioeconomic, and political reasons, together with increased international travel. New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening [10]. Brucellosis is a disease of worldwide distribution occurring in domestic as well as wild animals. It has been reported wherever animals are raised all over the world [19]. Although some of the industrialized countries in Europe and America have achieved the eradication of brucellosis in domestic animals through intensive control and eradication schemes, the disease is still a serious problem in developing countries [31,32].

B. melitensis is the most virulent species of the *Brucella* genus and the ones isolated most frequently in small ruminants in the Mediterranean, the Middle East and Latin America [33,34]. Brucellosis is a barrier to trade in animals and animal products and causes significant losses from abortion, as well as being a serious zoonosis [35-37].

Risk factors of brucellosis: The prevalence of brucellosis is influenced by several risk factors related to production systems, the biology of the individual host and environmental factors (climatic conditions and geography). These include species, sex, age, herd size and composition, the hygienic status of the farm, rate of contact between infected and susceptible animals, farm biosecurity and climate [2,11].

Brucellosis most frequently occurred in adult sheep and goats than the younger one [16]. Sexually mature and pregnant animals are more prone to *Brucella* infection and brucellosis than sexually immature animals of either sex [20,38]. Brucellosis of small ruminant's affects sexually matured animals; the predilection sites being the reproductive tracts of the males and females, especially the pregnant uterus. This may result from the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity [38].

Goats are at higher risk of acquiring *Brucella* infection than sheep. This may be due to the greater susceptibility of goats to *Brucella* infection. It could also be partly because goats excrete the organism for a long period, unlike sheep. This reduces the potential for diseases spread among sheep flocks [38]. The receptivity of ewes to *B. melitens* varies according to the breed. Milk producing ewes are more receptive than sheep raised for slaughter. Male animals are less susceptible to *Brucella* infection than females, due to the presence of a low concentration of erythritol in male relative to female animals [39].

Brucella may retain infectivity for several months in water, aborted fetuses and fetal membranes, feces and liquid manure, wool, hay, on buildings, equipment, and clothes. *Brucella* is also able to withstand drying particularly in the presence of extraneous organic material and will remain viable in dust and soil. *Brucella* is fairly sensitive to ionizing radiation and is readily killed by normal sterilizing doses of gamma-rays under conditions that ensure complete exposure, especially in colostrums [14].

Transmission: Generally, the transmission of small ruminant brucellosis occurs in the same way in sheep and goats as in cattle, materials excreted from the female genital tract forming the main supply of organisms for transmission to other animals and men. Therefore, in most circumstances, the primary route of dissemination of *Brucella* is the placenta, fetal fluids and vaginal discharges expelled by infected ewes after abortion or full-term parturition. Very large numbers of organisms are shed at the time of parturition or abortion. In goats, excretion of the organisms from the vagina is prolonged and copious (2 to 3 months generally). In sheep excretion is generally less prolonged, usually ceasing within 3 weeks after abortion or full-term parturition. Shedding of *Brucella* is also common in udder secretions and semen, and *Brucella* may be isolated from various tissues, such as lymph nodes from the head and those associated with reproduction, and sometimes from arthritic lesions [22].

Since *Brucella* species are intracellular pathogens of the animal hosts, the hosts are the reservoirs and can be the source of infection. Organisms reside inside cells of the reticuloendothelial system and reproductive tract and cause life long, chronic infections. Indeed, excretion of *Brucella* species only occurs at certain times, mainly when an abortion occurs. During an abortion, billions of *Brucella* species are excreted and this is a major source of infection for congeners and professionals in contact with aborted materials. The survival time of the organism outside the host is variable and depends on temperature and moisture. Colder weather extends survival time. Ingestion is the most common route of entry, although, exposure through the conjunctival and genital mucosa, skin and respiratory routes occur [40]. The infection is commonly transmitted from one ram to the other by perpetual contact. Transmission may also occur through the

ewe when an infected ram deposits his semen and another ram mates her shortly thereafter. The infection is not very common in ewes, and when it occurs it is contracted by sexual contact *B. ovis* does not persist very long in ewes and is generally eliminated before the next lambing period [41]. Only a small proportion of lambs and kids are infected *in vitro* and the majorities of B. melitensis latent infections are probably acquired through colostrums or milk [42].

Status of small ruminant brucellosis in Ethiopia: Brucellosis is a disease of many animal species including humans but especially of those that produce food animals: cattle, sheep, goats and other species [6,7]. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy and epididymitis and orchitis in the male. So the disease causes significant losses in the reproduction and productivity of sexually mature animals through high morbidity [2,10]. *B. melitensis*, *B. abortus,* and *B. suis* are zoonotic pathogens that can infect humans. *B. canis* may cause infections in immune-suppressed individuals.

Studies conducted on small-ruminant brucellosis in Ethiopia have indicated that seroprevalence of the disease is varied from place to place [43,44] which might be due to differences in animal production and management systems as well as reasonably difference in agro-ecological conditions of the study places (Table 1). Reports indicated that the prevalence of small-ruminant brucellosis was much higher in the area where farmers practice the communal use of grazing land than in clan-based flock/herd segregation areas [45]. This might be due to mixing animals from various areas in a communal grazing system and watering points.

Table 1: Reported prevalence of brucellosis in small ruminants in some areas of Ethiopia.

Location	Test used	Sample size	Overall prevalence (%)	References
Amibara District, Afar, Ethiopia	RBPT, CFT	226	12.4, 7.52	[48]
South East Somali and Oromia	RBPT	510	9.6	[49]
Bahir Dar, Northwest Ethiopia	RBPT, CFT	384	1.2, 0.4	[50]
East Showa, Oromia region	RBPT	384	1.56	[51]
Debreziet and Modjo export abattoirs	RBPT, CFT	853	1.99, 1.76	[52]
Gamo Gofa southern Ethiopia	RBPT, CFT	1000	4.3, 3.7	[53,54]

CFT: Complement Fixation Test
RBPT: Rose Bengal Plate Test

Treatment, prevention and control: One of the most successful methods for prevention and control of livestock brucellosis is through vaccination. In different parts of the world both live vaccines, such as *B. abortus* S-19, *B. melitensis* Rev1, *B. suis* S-2, rough *B. melitensis* strain M111 and *B. abortus* strain RB51 and killed vaccines, such as *B. abortus* 45/20 and *B. melitensis* H.38 are available. Use of the RB51 attenuated live vaccine has recently gained popularity for control of brucellosis in cattle [46].

Implementation of measures to reduce the risk of infection through personal hygiene, adoption of safe working practices, protection of the environment and food hygiene should minimize risks of further infection, the proper handling and burying of abortion materials to prevent contamination of water sources and pasture is of paramount importance. Furthermore, the common practice of feeding abortion materials to dogs should be avoided as this increases the risk of transmission to other animals. It is imperative to education on risks for infection to these populations in order to influence behavioral practices that will reduce risks of transmission [47].

The development of a national veterinary extension services in the country, is essential to promote awareness about brucellosis, its impact on livestock production and zoonotic risks, would provide a valuable prevention measure. This would help to unify both community/dairy animal producers to control and eliminate brucellosis. Currently, many dairy animal producers hide or dispose of animals with a history of abortion, potentially facilitating disease transmission between farms and regions. This seriously undermines efforts of controlling and preventing the disease [47].

Conclusion

Brucellosis is a worldwide distribution that causes the most important public wellbeing problem in developing country. It is the major public health problem and economically important disease in Ethiopia. Brucellosis is higher in pastoral rearing systems wherever human closely live with animals and so, are at higher probability to have brucellosis. Human is infected one there is contact with animals at abortion, parturition, or post parturition from splashing of infected droplets into the eye or drinking unpasteurized milk or milk product. Work-related exposure is observed in people who interaction with diseased animals or their tissue. This disease is the most important cause of reproductive inefficient and abortion in small ruminant. The occurrence of brucellosis is affected by factors related with production system, host and environmental factors. *B. melintesis* can be eradicated by isolated diseased animal, given vaccine for young female animals and test-slaughter methods. As a result, it is critical for conducting applicable control methods and increasing the community awareness on zoonotic transmission of brucellosis are suggested. In addition, further study should be carried out on epidemiology of the brucellosis in higher risk groups in Ethiopia.

References

1. Ethiopia Sheep and Goat Productivity Improvement Program. Control and Prevention of Common Reproductive Diseases of Sheep and Goats. 2011.
2. Radostits OM, Gay CC, Hinclcliff KW, Constable PO. Veterinary medicine: A text book of the disease of cattle, sheep, pigs, goat and horses. 10th ed. London: Saunders; 2007. p. 963-1110.
3. Akhvlediani T, Clark DV, Chubabria G, Zenaishvili O, Hepburn MJ. The changing pattern of human brucellosis: clinical manifestations, epidemiology and treatment outcomes over three decades in Georgia. BMC Infect Dis. 2010;10:346.
4. Roth F, Zinsstag J, Orkhon D, Chimed-Ochir G, Hutton G. Human health benefits from livestock vaccination for brucellosis: case study. Bull World Health Organ. 2003;8(1):867-76.
5. Robinson A. Guidelines for coordinated human and animal brucellosis surveillance. FAO Animal Production and Health paper 156. Rome: FAO, Italy; 2003. p. 1-45.
6. Corbel MJ. Brucellosis in humans and animals. World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organization for Animal Health. 2006.
7. Ghanem YM, El-Khodery A, Saad A, Abdelkadir H, Heybe A, Musse A. Seroprevalence of camel brucellosis (Camelis dromedarus) in Somaliland. Trop Anim Health Prod. 2009;41:1779-86.
8. WHO. Brucellosis in humans and animals. Geneva: World Health Organ; 2006. p. 27-66.
9. OIE. Caprine and Ovine Brucellosis (Excluding Brucella ovis), In: OIE (ed). Manual

of Diagnostic Tests and Vaccines for Terrestrial Animals 2009, Vol. OIE-World Organization for Animal Health, Office International des Epizooties. 2009. p. 2-8.

10. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis. 2006;6:91-9.

11. McDermont JJ, Arimi SM. Brucellosis in sub-Saharan Africa: Epidemiology, Control and Impact. Vet Microbiol. 2002;20:111-34.

12. Alebachew T, Adane T, Arega T, Tsehaye H, Ayichew T. Review on Small Ruminant Brucellosis in Ethiopia. Adv Life Sci Technol. 2016;46:16-24.

13. Vassalo DJ. The corps disease. Brucellosis and its historical association with the Royal Army Medical Corps. J R Army Med Corps. 1992;138:148-50.

14. Glenn JS, Karen WP. Veterinary Microbiology: Bacterial and Fungal agents of animal diseases. 1st ed. Philadelphia: Saunders publishers; 2005. p. 200-3.

15. Poester FP, Nielsen K, Samartino LE, Yu WL. Diagnosis of brucellosis. Open Vet Sci J. 2010;4:46-60.

16. Walker RL. Veterinary Microbiology. Black wells Science, Cambridge, Massachusetts. 1999. p.196-203.

17. Tabak F, Hakko E, Mete B, Ozaras R, Mert A, Ozturk R. Is family screening necessary in Brucellosis? Infect. 2008;36(6):575-7.

18. Pizarro J, Meresse S, Parton RG. Brucella abortus transits through the autophagic path way and replicates in the endoplasmic reticulum of non-professional phagocytes. Infect Immun. 1998;66(12):5711-24.

19. Seifert HSH. Diseases caused by aerobic rods. I. Brucellosis. In: Tropical Animal Health. Kluwer Academic Publishers. 1996. p. 356-67.

20. Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology. Ireland. p. 261-7.

21. Godfroid J, Nielsen K, Saegerman C. Diagnosis of brucellosis in livestock and wildlife. Croat Med J. 2010;51:296-305.

22. Alton GG. Brucella melitensis. In: Nielsen, H.K and Duncan, J.R. (Eds). Animal brucellosis. Buca Raton: CRC Press; 1990. p. 383-409.

23. Macmillan AP. Conventional serological tests. In: Animal brucellosis. Florida: CRC press; 1990. p. 153-90.

24. European Commission. Brucellosis in sheep and goats. Report of Scientific Committee on Animal Health and Animal Welfare Directorate General for Health and Consumers, Brussels; 2001. p. 10-46.

25. OIE. Manual of the diagnostic tests and vaccines for terrestrial animals, 5th Ed. France: Office International des Epizooties; 2004. p. 409-38.

26. Plumb GE, Olsen SC, Buttke D. Brucellosis: 'One Health' challenges and opportunities. Rev Sci Tech. 2013;32(1):271-8.

27. Garin B, Blasco JM. Caprine and ovine brucellosis (excluding B. ovis). In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th OIE. 2004. p. 598-606.

28. Blasco JM, Garin B, Marin CM, Gerbier G, Fanlo J, Bagues M, et al. Efficacy of differentiating Rose Bengal and Complement Fixation antigen for diagnosis of Brucella melitensis in sheep and goats. Vet Rec. 1994;134:415-20.

29. Ferreira AC, Cardozo R, Travassos I, Mariano I, Belo A, Rolao Preto I, et al. Evaluation of a modified Rose Bengal test and an indirect enzyme-linked immune-sorbent assay for diagnosis Brucella melitensis infection in sheep. Vet Res. 2003;34(3):297-305.

30. Garin B. The diagnosis of brucellosis in sheep and goats, old and new tools. Small Ruminant Research. 2006;62:63-70.

31. Warner D. Brucellosis in animals and man. In: Goodman DE, editor. Zoonosis: animal disease that affect man. Christian Vet Miss. Seattle. 2001. p. 23-32.

32. Ragan VE. The Animal and Plant Health Inspection Services. brucellosis eradication program in the United States. Vet Microbiol. 2002;90(1-4):11-8.

33. Lucero NE1, Ayala SM, Escobar GI, Jacob NR. Brucella isolated in humans and animals in Latin America from 1968 to 2006. Epidemiol Infect. 2008;136(4):496-503.

34. Blasco JM, Molina B. Control and eradication of Brucella melitensis infection in sheep and goats. Vet Clin North Am Food Anim Pract. 2011;27(1):95-104.

35. Benkirane A. Ovine and caprine brucellosis world distribution and eradication strategies in West Asia and North Africa region. Small Ruminant Res. 2006;62(1):19-25.

36. Banai M. Control of Brucella melitensis. Memorias del IV Foro Nacional de Brucelosis, Facultad de Medicina Veterinariay Zootecnia de la Universidad Nacional Autónoma de México (FMVZ-UNAM). 2007;26-7.

37. Seleem MN, Boyle SM. Sriranganathan N. Brucellosis: re-emerging zoonosis. Vet Microbiol. 2010;140(3-4):392-8.

38. Radostits OM, Gay CC, Blood CD, Hinchcliff KW. Veterinary Medicine, Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses. 9th ed. New York: WB Saunders; 2000. p. 867-82.

39. Hirsh DC, Zee YC. Veterinary Microbiology. Blackwell Science. Cambridge: Massachusetts; 1999. p. 196-203.

40. Dwight CH, Yaun CZ. Veterinary Microbiology: Blackwell Science. Cambridge: Massachusetts; 1999. p. 197-201.

41. PAHO. Zoonoses and communicable diseases which is common to man and animals. 3rd ed. Am Health Organ; 2001.

42. Grilló MJ, Barberán M, Blasco JM. Transmission of Brucella melitensis from sheep to lambs. Vet Rec. 1997;140(23):602-5.

43. Ashagirie T, Deneke Y, Tolosa T. Seroprevalence of caprine brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia. Afr J Microbiol Res. 2011;5(13):1682-5.

44. Bekele M, Mohammed H, Tefera M, Tolosa T. Small ruminant brucellosis and community perception in Jijiga district, Somali Regional State, Eastern Ethiopia. Trop Anim Health Prod. 2011;43(4):893-8.

45. Yibeltal M. A Seroprevalence Study of Small Ruminant Brucellosis in Selected Sites of the Afar and Somali Regions, Ethiopia DVM Thesis Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia; 2005.

46. Cheville NF, Olsen SC, Jensen AE, Stevens MG, Palmer MV, Florance AM. Effects of age at vaccination on efficacy of Brucella abortus strain RB51 to protect cattle against brucellosis. Am J Vet Res. 1996;57(8):1153-6.

47. Yohannes M, Degefu H, Tolosa T, Belihu K, Cutler R, Cutler S. Brucellosis in Ethiopia. Afr J Microbiol Res. 2013;7(14):1150-7.

48. Muluken T. Seroprevalence of Brucellosis and Isolation of Brucella from small ruminants that had history of recent abortion in selected Kebelesof Amibara District, Afar Region, Ethiopia. M.Sc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia. 2016.

49. Gumi B, Firdessa R, Yamuah L, Sori T, Tolosa T. Seroprevalence of Brucellosis and Q-Fever in Southeast Ethiopian Pastoral Livestock. J Vet Sci Med Diagn. 2013;2(1):1-5.

50. Yeshwas F, Desalegne M, Gebreyesus M, Mussie HM. Study on the seroprevalence of small ruminant brucellosis in and around Bahir Dar, North West Ethiopia. Ethiopian Vet J. 2011;15(2):35-44.

51. Lemu D, Mamo H, Deressa A, Pal M. A study on seroprevalence of brucellosis in goats and sheep in East Shewa, Ethiopia. Ethiopian Int J Multidiscip res. 2014;1(4):14-8.

52. Tsegay A, Tuli G, Kassa T, Kebede N. Seroprevalence and risk factors of Brucellosis in small ruminants slaughtered at Debre Ziet and Modjo export abattoirs, Ethiopia. J Infect Dev Ctries. 2015;9(4):373-80.

53. Melese Y. Isolation, phenotype characterization and Seroprevalence Surevy on small ruminant's brucellosis in Arba Minch Zuria and Mirab Abaya districts of Gamo Gofa, Southern Ethiopia. 2016.

54. D'anastasio R, Staniscia T, Milia ML, Manzoli L, Capasso L. Origin evolution and paleoepidemiology of brucellosis. Epidemiol Infect. 2011;139(1):149-56.