



A Review on Brucellosis in Small Ruminants

Wogayehu Seria*, Yosefdeneke Diriba Tadese, Eshetu Shumi

College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia

Email address:

Yosefdeneke@yahoo.com (W. Seria)

*Corresponding author

To cite this article:

Wogayehu Seria, Yosefdeneke Diriba Tadese, Eshetu Shumi. Review on Brucellosis in Small Ruminants. *American Journal of Zoology*. Vol. 3, No. 1, 2020, pp. 17-25. doi: 10.11648/j.ajz.20200301.14

Received: August 19, 2019; **Accepted:** September 24, 2019; **Published:** February 28, 2020

Abstract: *Brucellamelitensis* is the main causative agent of caprine and ovine brucellosis. Sporadic cases caused by *B. abortus* have been observed, but cases of natural infection are rare in sheep and goats. Brucellosis is an infectious disease of many domestic and wild animals. Brucellosis is a major cause of direct economic losses resulting from clinical disease, abortion, neonatal losses, reduced fertility, decreased milk production, emergency slaughtering of the infected animals and treatment costs. It also plays a significant role as a barrier for international trade of live animals by being used as an impediment to free animal movement and export. Economic losses in small ruminants stem from breeding inefficiency, loss of lambs and kids, reduced wool, meat and milk production. Clinically, the disease is characterized by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis, with excretion of the organisms in uterine discharges and in milk. Diagnosis depends on the isolation of *Brucella* from abortion material, udder secretions or from tissues removed at post-mortem. Presumptive diagnosis of *Brucella* infection can be made by assessing specific cell-mediated or serological responses to *Brucella* antigens. *Brucellamelitensis* is highly pathogenic for humans, causing Malta fever, one of the most serious zoonoses in the world. Identification of the agent Presumptive evidence of *Brucella* is provided by the demonstration, by modified acid-fast staining of organisms typical of *Brucella* in abortion material or vaginal discharge, especially if supported by serological tests. The polymerase chain reaction (PCR) methods provide additional means of detection.

Keywords: Abortion, Brucellosis, Diagnosis, Jimma, Small Ruminant, Zoonosis

1. Introduction

In Ethiopia, small ruminants have been reared for long time for similar purposes. They have their own contribution to the country's economy. This is not because they are productive but are huge in number. It is quite evident that small ruminant farms in Ethiopia are extensive type of production occupied by individual farmers and consequently, the outcome as a whole is below expectation and limited. Constraints which restrict the potential of extensive farms of small ruminants in Ethiopia include the presence of diseases of various natures, poor management, lack of appropriate selection and breeding and low input of feeding [1].

It is a growing concern, however, that there is expansion of diseases various etiologies in to our small ruminant animals. Among various bacterial diseases of small ruminants brucellosis is the most economically important diseases of ruminants [1].

Brucellosis is an infectious bacterial disease caused by members of the genus *Brucella*. It is disease of worldwide importance and affects a number of animal species. Species of *Brucella* are obligate parasites, requiring an animal host for maintenance. Members of genus *Brucella* species are the agents of the brucellosis, a worldwide zoonotic disease. The host range includes humans, ruminants, swine, rodents, canines and marine mammals. Infection occurs through inhalation or ingestion of organisms. High numbers of the organism are shed in urine, milk, vaginal discharge, semen and through discharges of birth. Under appropriate conditions, *Brucella* can survive outside the host in the environment for extended periods. They may remain viable in carcass and tissues for 6 months at 0°C up to 125 days in soil, and as long as [1] year in feces [2]. In active cause of brucellosis of small ruminants can be diagnosed by isolation and identification of the responsible micro-organisms using bacteriological tests which determine the

phenotypic characteristics of the bacteria. *Brucella* can also be detected using molecular tests which take account of all the characteristics of the genome. But in chronic infection the disease is diagnosed by different immunological (allergic test) and serological tests that can be screening and confirmatory serological tests [3].

Research and case reports revealed that small ruminant brucellosis is the major health constraints inflicting heavy losses in small ruminant's production systems in Ethiopia along with other bacterial and viral diseases of ruminants [1].

Therefore, the objectives of this seminar are:

- (i) To highlight the Epidemiology and pathogenesis of small ruminant brucellosis.
- (ii) To understand available diagnostic techniques for small ruminants brucellosis and to assess their merits and limitations.

2. Literature Review

Brucellosis of small ruminants is an infectious disease of goat and sheep characterized by mass abortion in ewes, epididymitis and orchitis in rams in first out breaks of disease. However it becomes chronic and latent after the first outbreak of the disease [4].

Etiology

Brucellosis in sheep and goats (excluding *Brucella ovis* infection) is primarily caused by one of the three biovars of *B. melitensis* and *B. abortus*. *B. melitensis* is most commonly infects sheep and goats. The organism is regarded as the most virulent of the *Brucella* species and accounts for most cases of human brucellosis. Breed susceptibility is variable in sheep, but goat breeds are highly susceptible. *B. ovis* primarily affects rams [2].

Pathologically and epidemiologically, *B. melitensis* infection in sheep and goats is very similar to *B. abortus* infection in cattle. *Brucellamelitensis*, *B. abortus* and *B. ovis* are small, non - motile, non - sporulating, non toxigenic, aerobic, facultative intracellular, gram-negative coccobacilli parasites, and based on DNA homology it represents a single species (Moreno, 2002; Grimont *et al.*, 1992). Taxonomically, genus *Brucella* is classified as a *Proteobacteria* and subdivided into six species, each comprising several behaviors [5].

Species of *Brucella* grow best on tripticase soya based media or other enriched media like blood agar. Species of *Brucella* may produce urease and may oxidize nitrite to nitrate; they are oxidase and catalase positive. Species and biovars are differentiated by their carbon dioxide requirements; ability to use glutamic acid, ornithine, lysine, and ribose; production of hydrogen sulphide; growth in the presence of thionin or basic fuchsin dyes; agglutination by anti-sera directed against certain lipopolysaccharide (LPS) epitopes; and susceptibility to lysis by bacteriophages. Analysis of fragment lengths of DNA cut by various restriction enzymes has also been used to differentiate *Brucella* groupings [4].

3. Epidemiology

3.1. Geographic Distribution and Transmission

Brucellamelitensis is particularly common in the Mediterranean. It also occurs in the Middle East, Central Asia, around the Persian Gulf (also known as the Arabian Gulf), and in some countries of Central America. This organism has been reported from Africa and India, but it does not seem to be endemic in northern Europe, North America (except Mexico), Southeast Asia, Australia, or New Zealand. Biovar 3 is the predominant biovar in the Mediterranean countries and the Middle East, and biovar 1 predominates in Central America. Sporadic cases or incursions are occasionally reported in *B. melitensis* free countries. In the U.S., cases have mainly been reported in imported goats and rarely in cattle [6].

Brucellosis is an infectious disease of many domestic and wild animals. Abortion is the most important symptom in the later stages of pregnancy. In most circumstances, the primary route of transmission of species of *Brucella* is the placenta, fetal fluids and vaginal discharges expelled by infected ewes and goats when they abort or have 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 heart, spleen and organs associated with reproduction (uterus, epididymides and testes), and from arthritic lesions [7]. The receptivity of ewes to *B. melitensis* varies according to the breed. Milk producing ewes are more receptive than sheep raised for slaughter. Since the bacteria are intracellular pathogens of the animal hosts, these hosts are the reservoirs of and can be the source of infection. Organisms reside inside cells of reticulo-endothelial system and reproductive tract and cause life long, chronic infections. Indeed, excretion of *Brucella* species only occurs at certain times, mainly when abortion occurs. During an abortion, billions of *Brucella* species are excreted and this is a major source of infection for congeners and for professionals in contact with aborted materials. Survival time of the organism outside the host is variable and depends on temperature and moisture. Ingestion is the most common route of entry, although, exposure through the conjunctival and genital mucosa, skin and respiratory routes occurs [8].

In ram epididymitis caused by *B. ovis*, semen is the main and possibly the only source of infection. The infection is commonly transmitted from one ram to the other by preputial 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 [9]. However, only a small proportion of lambs and kids are infected "in vitro" and the majority of *B. melitensis* latent infections are probably acquired through colostrum or milk [7]. Despite the low frequency of transmission, the existence of such latent infections increases the difficulty of eradicating this disease, as the bacteria persist in the animal without inducing

detectable immune responses. The exact mechanism of the development of *B. melitensis* latent infections remains unknown [10]. In many parts of the world, small ruminants and cattle (and frequently also camels, yaks and buffaloes) are reared together. In these production systems the existence of cross-infections is very frequent with *B. melitensis* being the most common cause of infection when the above animal species are reared together. *Brucellosis* is readily transmissible to humans, causing acute febrile illness undulant fever which may progress to a more chronic form and can also produce serious complications affecting the musculoskeletal, cardiovascular, and central nervous systems [11].

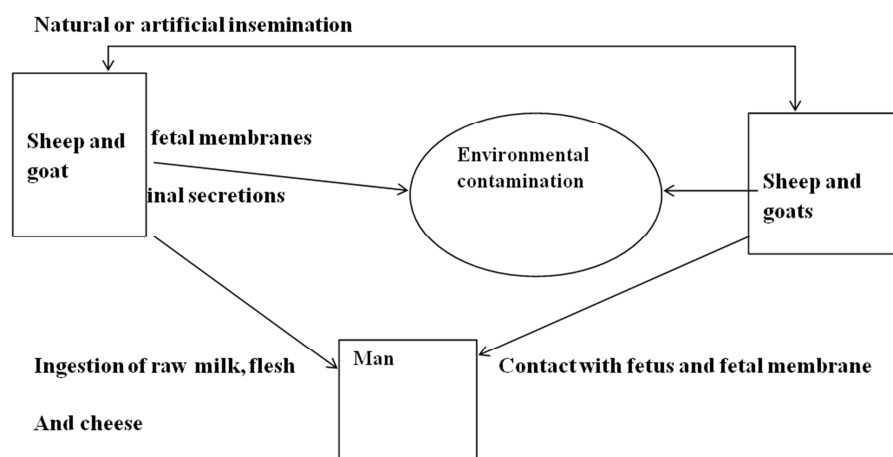
3.2. Source Infection in Human

The most common sources of infection for humans are unpasteurized milk and milk products, occupational contacts (farmers, veterinarians, slaughter house workers and so on) with infected materials. *Brucellosis* is rarely, if ever, transmitted from person to person. The incidence of human disease is thus closely tied to the prevalence of infection in sheep, goats, and cattle, and to practices that allow exposure of humans to potentially infected animals or their products. *B. melitensis* infection of small ruminants is quite similar in both pathological and epidemiological standpoints to *B. abortus* infection of cattle. Infection is often due to occupational exposure and is essentially acquired by the oral, respiratory, or conjunctival routes, but ingestion of dairy products constitutes the main risk to the general public. There is an occupational risk to veterinarians, abattoir workers and

farmers who handle infected animals and aborted fetuses or placentas. *Brucellosis* is one of the most easily acquired laboratory infections, and strict safety precautions should be observed when handling cultures and heavily infected samples, such as products of abortion. The most reliable and the only unique method for diagnosing animal brucellosis is isolation of *Brucella* species [11].

Animals may transmit *Brucella* organisms during septic abortion, during slaughter, and through their milk. The most common sources of infection for humans are unpasteurized milk and milk products, occupational contacts (farmers, veterinarians, slaughter house workers and so on) with infected materials [7].

Brucellosis is rarely, if ever, transmitted from person to person. The incidence of human disease is thus closely tied to the prevalence of infection in sheep, goats, and cattle, and to practices that allow exposure of humans to potentially infected animals or their products. *B. melitensis* infection of small ruminants is quite similar in both pathological and epidemiological standpoints to *B. abortus* infection of cattle. Only when the animals excrete the bacterium do they become dangerous to other animals and human beings. In most circumstances, the primary (and more relevant from the epidemiological standpoint) excretion of *B. melitensis* is the placenta, fetal fluids and vaginal discharges expelled by infected animals after abortion or full-term parturition. Shedding of *B. melitensis* is also common in udder secretions and semen. *Brucella* may be isolated from various tissues, such as lymph nodes from the head and those associated with reproduction, and from arthritic lesions [7].



Source: (PAHO, 2001).

Figure 1. Mode of transmission of small ruminants' brucellosis.

3.3. Morbidity and Mortality

Brucella melitensis is a significant problem in small ruminants, particularly in developing nations where infections can be widespread. The relative importance of *B. melitensis* for sheep and goats varies with the geographic region, and can be influenced by husbandry practices and the susceptibility of sheep breeds in the region. Management practices and environmental conditions significantly

influence the spread of infection. Lambing or kidding in dark, crowded enclosures favors the spread of the organism, while open air parturition in a dry environment results in decreased transmission. The abortion rate is high when *B. melitensis* enters a previously unexposed and unvaccinated flock or herd, but much lower in flocks where this disease is enzootic. Ruminants usually abort only during the gestation when they are first infected. Inflammatory changes in infected mammary glands usually reduce milk yield by a minimum of

10%. Fertility in males can be permanently impaired. Deaths are rare except in the fetus [12].

4. Pathogenesis

Brucellamelitensis can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, the gastrointestinal tract and through reproductive tracts. In the alimentary tract the epithelium covering the ileal Peyer's patches are preferred site for entry. In the gastrointestinal tract, the organisms are phagocytosed by lympho-epithelial cells of gut-associated lymphoid tissue, from which they gain access to the sub-mucosa [11]. Infections tend to be localized to the reticuloendothelial system and genital tract with abortion in females, and epididymitis and orchitis in males are the common clinical manifestations. Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them and are also phagocytosed by macrophages. Bacteria transported in macrophages, which travel to lymphoid tissue draining the infection site, may eventually localize in lymph nodes, liver, spleen, mammary glands, joints, kidneys, and bone marrow. In macrophages, *B. melitensis* inhibits fusion of phagosome and lysosome (Harmon *et al.*, 1988) and replicate within compartments that contain components of endoplasmic reticulum [13]. via a process facilitated by the type IV secretion system. If unchecked by macrophage bactericidal mechanisms, the bacteria destroy their host cells and infect additional cells. *Brucella* can also replicate extracellularly in host tissues [14].

Histopathological, the host cellular response may range from abscess formation to lymphocytic infiltration to granuloma formation with caseous necrosis. Serum complement effectively lyses some rough strains (i.e. those that lack O-polysaccharide side chains on their LPS), but has little effect on smooth strains (i.e. bacteria with a long O-polysaccharide side chain); *B. melitensis* may be less susceptible than *B. abortus* to complement-mediated killing [15]. These observations suggest that *Brucella*, like other facultative or obligate intra-macrophage pathogens, are primarily controlled by macrophages activated to enhanced microbicidal activity by IFN- γ and other cytokines produced by immune T lymphocytes. It is likely that antibody, complement, and macrophage-activating cytokines produced by natural killer cells play supportive roles in early infection or in controlling growth of extracellular bacteria. 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 [16]. In the absence of effective intracellular microbicidal mechanisms, these tissues permit exuberant bacterial growth, which leads to fetal death and abortion. In ruminants, the presence in the placenta of erythritol may further enhance growth of *Brucella*. Exudates and discharges at the time of abortion may contain up to 10^{10} bacteria per gram of tissue. When septic abortion occurs, the intense concentration of bacteria and aerosolization of

infected body fluids during parturition often result in infection of other animals and humans [17].

5. Clinical Sign and Finding

The main clinical manifestations of brucellosis in sheep and goats are, as in all female ruminants, reproductive failure, abortion and birth of weak offspring. Abortion generally occurs during the last two months of pregnancy and is followed in some cases by retention of fetal membranes. In the male, localization in the testis, epididymis and accessory sex organs is common, and bacteria may be shed in the semen. This may result in acute orchitis and epididymitis and later in infertility. Arthritis is also observed occasionally in both sexes [18].

Animals generally abort once, although reinvasion of the uterus occurs in subsequent pregnancies and organisms are shed with the membranes and fluids. Non-pregnant animals exposed to small numbers of organisms may develop self-limiting, immunizing infections or they may become latent carriers. Persistent infection of the mammary glands and supra mammary lymph nodes is common in goats with constant or intermittent shedding of the organisms in the milk in succeeding lactations, while the self-limiting nature of the disease in sheep, which is seldom accompanied by prolonged excretion of the bacteria, has been observed [19].

The inflammatory changes in the infected mammary gland reduce milk production by an estimated minimum of 10%. Orchitis and epididymitis generally lead to a chronic infection. Infected animals generally develop granulomatous inflammatory lesions which frequently are found in lymphoid tissues and organs such as reproductive organs, udder and supra mammary lymph nodes and sometimes joints and synovial membranes. This disease has no pathognomonic lesions and the changes that can be observed are necrotizing placentitis, palpable testicular alterations, necrotizing orchitis and epididymitis with subsequent granuloma, necrotizing seminal vesiculitis and prostatitis. Some aborted fetuses may have an excess of blood-stained fluids in the body cavities, with enlarged spleen and liver. Others appear normal. Infected fetal membranes show changes affecting part or all of the membrane. The necrotic cotyledons lose their blood-red appearance becoming thickened and dull-grey in color. In the chronic stage of the disease the epididymis can be increased in size up to four or fivefold [12].

6. Diagnostic Methods of Brucellosis in Small Ruminants

In active cause of brucellosis of small ruminants can be diagnosed by isolation and identification of the responsible micro-organisms using bacteriological tests which determine the phenotypic characteristics of the bacteria. *Brucella* can also be detected using molecular tests which take account of all the characteristics of the genome. But in chronic infection the disease is diagnosed by different immunological (allergic

test) and serological tests that can be screening and confirmatory serological tests [3].

6.1. Bacterial Isolation and Identification

6.1.1. Specimen Collection

In abortion cases a full range of specimens should be collected and submitted for a differential diagnosis. A whole fetus can also be sent if it is feasible. Alternatively, uterine discharges, fetal stomach contents, any fetal lesions, cotyledons, colostrum's, paired serum samples, and sections of cotyledons and fetal lesions can be collected aseptically for bacterial isolation and identification. The most valuable specimens for bacterial culture are aborted fetal tissues (especially lung, spleen, and stomach contents), placenta, lymph nodes, post parturient uterus, vaginal discharge, semen, urine and bone marrow. All specimens must be packed separately and transported immediately to the laboratory in ice box with ice packs in leak proof containers). If the specimens are not inoculated immediately, preserve in refrigerator at 4°C [2]. Additionally, the above specimens must be preserved 10% formalin for histopathology. Semen and tissue from epididymitis or testes from males could be examined [4]. Recommended samples for bacteriological examination of aborted male and female [7].

6.1.2. Direct Microscopic Examination from Specimens

Smears are made from specimens and stained by modified Ziehl-Neelsen (MZN) stain. *Brucella* appears as small, red-staining coccobacilli in clumps because of their intracellular growth. In Gram staining they appear gram negative coccobacilli in clumps [3].

6.1.3. Isolation of Pure Colony and Pure Culture

Isolation of pure colony of *B. melitensis* can be done from the above specimens and from blood or bone marrow from the sternum or ileal crest taken while the patient is febrile. The pure colony of bacteria can be obtained by streaking the specimens on appropriate media. Culture material may also be taken from lymph nodes, cerebrospinal fluids, and abscesses. It is recommended the cultures be repeated several times to get pure colony then pure culture. The bacteria grow on selective blood, serum dextrose, tryptose soya and *Brucella* albumin agars. If contamination is likely to be a problem, attempt for isolation should be made using media containing actidione 30mg/l, bacitracin (7500µg/l) and polymyxin (1800µg/l). There are also selective media which are used both with and without the incorporation of ethyl violet (1:800,000) [9].

Tissues are cultured directly on solid media. Milk cultures are performed by centrifuging milk at 5900 to 7700 rpm for 15 minutes or by allowing for gravity cream separation to occur over night. Both the cream layer and the sediment if the centrifugation technique is used should be streaked on solid media. Culture should be incubated at 37°C in 10% CO₂ for a minimum of 10 days in highly suspicious cases. Animal inoculation is the most sensitive method for detection of *Brucella* and is sometimes necessary when very low

numbers of organisms are present. Two guinea pigs are inoculated and at 3 and 6 weeks post inoculation an animal is sacrificed [8].

6.1.4. Identification

Identification of the bacteria begins with colony morphology of the pure colony and culture. The colonies of *B. melitensis* on selective media contain a blood agar base with 5% sterile sero negative equine or bovine serum and an antibiotic supplement (Polymyxin B sulphate, bacitracin, Cycloheximide and Nalidixic) are non haemolytic and are usually smooth form in the first isolate and they become rough when they are sub cultured [8].

For routine identification *Brucella*, colonial morphology, staining properties and a few biochemical tests are performed. Preliminary identification of *Brucella* species requires microscopic examination of pure culture; and shows they are Gram-negative coccobacilli; and are non-motile, catalase positive, oxidase positive (except *B. ovis* and *B. neotomae*) and give rapid urease activity (except *B. ovis* and some strains of *B. melitensis*). They reduce nitrate and are indole negative [3].

Some biochemical tests that must be performed to differentiate species of *Brucella* are H₂S production; urease activity in hours; growth in the presence of dye thionin (20 µg/ml) and dye basic fuchsin (20 µg/ml). Definitive identification is usually performed by *Brucella* reference laboratory. A fluorescent antibody test is used for rapid identification (Dwight and Yuan, 1999). After species identification, it is important to do biotype determination. The differentiation of biotype of the six main species of *Brucella* is achieved by four tests that are requirements for CO₂, production of H₂S, growth in the presence of stains and agglutination by mono specific sera [8]. Requirement for CO₂ determines the absolute requirement of the developing culture for CO₂. It must be carried out after isolation at the time of transfer for selective medium to non-selective medium for purity control. Hydrogen sulphide production for *Brucella* is determined using strips of paper impregnated with lead acetate. The principle of the test is that when a strip produces hydrogen sulphide, the sulfur combines with the lead to form a black precipitate of lead acetate in the strips. The different *Brucella* species are sensitive or resistant to thionin or basic fuchsin incorporated directly in to the base media. Agglutination with mono-specific sera is used to determine the *B. melitensis* types. It is the only test that differentiates the three biotypes of this species. The principle of the test is that all smooth *Brucella*, independent of species, possess two determinant surface antigens, A and M, distributed in different proportions according to the strain. Characterization of the three possible antigens profiles lies in a simpler plate agglutination test using specific sera of A and M antigens. A dominant strains are only agglutinated by anti-A sera, M-dominant strains by anti-M sera and strains containing notable quantities of both epitopes by both sera [7].

Most species of *Brucella* shares common antigens. The common antigens of species of *Brucella* are the abortus and melitensis antigens. The melitensis antigen is the most virulent antigen. The above common antigenic structure exist with different proportions as it is illustrated.

6.2. Serological Test

6.2.1. Rose Bengal Plate Test (RBPT)

The Rose Bengal Plate Test (RBPT) is the most common screening test for detection of *Brucella* agglutinins. The principle of the test is that the sera collected from animals were mixed with antigen and examined for agglutination [12]. The use of the Rose Bengal Plate Test, which is easy to perform and is considered a valuable screening test, is less effective than the CFT at detecting brucellosis in small ruminants. Buffered plate agglutination (BPA) tests are the well-known buffered *Brucella* antigen tests. These tests are rapid agglutination tests lasting 4 minutes and it is done on a glass plate with the help of an acidic-buffered antigen (pH 3.65 ± 0.05). These tests have been introduced in many countries as the standard screening test because it is very simple and thought to be more sensitive than the SAT20.

6.2.2. Milk Ring Test

Another screening test used to diagnose brucellosis is milk ring test. It consists of mixing colored *Brucella* whole-cell antigen with fresh bulk/tank milk. In the presence of anti *Brucella* antibodies antigen - antibody complexes form and migrate to the cream layer, forming a purple ring on the surface. In the absence of antigen-antibody complexes, the cream remains colorless. This test is not considered sensitive but this lack of sensitivity is compensated by the fact that the test can be repeated, usually monthly, due to its very low cost. This procedure is valuable in screening dairy cows and has limitations in the diagnosis of caprine and ovine brucellosis. The smaller fat globulin of goat and sheep cream absorbs agglutinated stained *Brucella* in positive milk samples less efficiently and do not rise to form the typical [7]. colored ring. An additional problem with the MRT is the low content of antibodies in goat and sheep milk. The sensitivity of the MRT can be increased by performing it in hypertonic medium of 5% NaCl. A serious disadvantage of the test is that its use is limited to milking animals. The milk ring test is based on agglutination of antibodies secreted into the milk.

The advantage of milk ring test is its ability to detect antibodies in the milk. However, it has limitation because it detects milk antibodies only on lactating animals, very uncertain at individual animal level and only applicable on entire herd, yields a rough picture of the status of the infection there is also screening test which is card test. This method is the most suitable for detecting infected flocks and used mostly for survey. It is simple and rapid and does not require laboratory facilities [22].

6.2.3. Enzyme Linked Immune Sorbent Assay

Another confirmatory serological test used for brucellosis is Enzyme linked Immune (ELISA). Since neither a single

serological test nor combined infected animals in a flock, detection of brucellosis remains a major problem in areas of low prevalence of Brucellosis. Most studies agree that the ELISA is as specific as the CFT but it is more sensitive. Yet, for a reliable diagnosis of infected animals studies suggest using the ELISA in combination with other tests. Small ruminants should be tested with the ELISA and CFT tests to prevent the spread of brucellosis after an outbreak of the disease in an area with low prevalence of brucellosis or in an area free from brucellosis [22].

7. Significance on Economic and Public Health

7.1. Public Health Significance

Since there is close contact between humans and their livestock, which sometimes share the same housing enclosures, brucellosis is a significant health risk for the entire community. It is readily transmissible to humans, causing acute febrile illness undulant fever which may progress to a more chronic form and can also produce serious complications affecting the musculoskeletal, cardiovascular, and central nervous systems. Brucellosis is a zoonotic bacterial disease caused by *Brucella* spp. and is primarily a disease of animals whereas humans are accidental hosts [23]. The disease is one of the most widespread zoonotic and is endemic in many countries. It is also considered a neglected zoonotic by the WHO [24].

There are six identified species and numerous biotypes. *B. melitensis* bacteria show a strong host preference although cross-species infections happen, particularly with *B. melitensis*. Clinical manifestation among humans is acute febrile illness which may persist and develop into a chronic disease with serious complications, such as joint illness, organ failure and symptoms of mental illness. The mortality rate is relatively low, especially when the patient is treated with adequate antibiotics; however this is not the case for everyone in low income countries [23].

In endemic countries humans get infected mainly by drinking unpasteurized milk and/or exposure to aborted fetuses, placentas or infected animals [25]. There is an occupational risk to veterinarians, abattoir workers and farmers who handle infected animals and aborted fetuses or placentas. Brucellosis is one of the most easily acquired laboratory infections, and strict safety precautions should be observed when handling cultures and heavily infected samples, such as products of abortion. The most reliable and the only unique method for diagnosing animal brucellosis is isolation of *Brucella* species [11].

7.2. Economic Importance

Brucellosis presents a significant impediment to the economic potential of the large population of small ruminants. Since small ruminants and their products is an important export commodity, detaining seropositive animals in

quarantine has a negative economic impact. The main economic consequences of brucellosis in small ruminants are: infertility, a high mortality in lambs and kids, outbreak, vaccine and research costs, movement restrictions, culling, market loss due to risk of infected meat, and milk, mortality, morbidity, lower production, loss of exports, loss of animal genetic resources and opportunities occasioned by spending on disease prevention and, mastitis. The reproductive wastage associated with brucellosis is another obstacle to optimal exploitation of the small ruminant sector. Reproductive losses are due to abortion, birth of weak offspring, and infertility [3].

7.3. Status of Small Ruminants Brucellosis in Ethiopia

Studies conducted on small-ruminant brucellosis in Ethiopia have indicated that sero-prevalence of the disease is varied from place to place [26] which might be due to the differences in animal production and management systems as well as reasonably difference in agro-ecological conditions of the study places and C. Reports indicated that the prevalence of small animal ruminant brucellosis was much higher in area where farmers practice the communal use of grazing land than in clan-based flock/herd segregation areas. This might be due to mixing animals from various areas in communal grazing system and watering points. Reported prevalence proportion of 1.5% in sheep and 1.3% in goats in the central highlands, 15% in sheep and 16.5% in goats in the Afar region, 1.6% in sheep and 1.7% in goats in the Somali region [27]. And 1.6% in sheep and 1.7% in goats in Somali region [28].

7.4. Control and Prevention Strategies

7.4.1. Vaccination

Control of brucellosis can be achieved by using vaccination to increase the population's resistance to the disease. Vaccination practically eliminates the clinical signs of brucellosis and is accompanied by a reduced contamination of the environment as well as exposure of the population at risk to the infectious agent [29]. The *B. melitensis* REV 1 vaccine is an attenuated strain of *B. melitensis* and an effective method to reduce the prevalence of brucellosis among whole flocks or herds in low income countries and/or endemic countries [21], [23]. However, in many countries, where the animals were kept under extensive conditions with nomadic or semi-nomadic husbandry, this approach was impractical and failed to reduce the incidence and prevalence of the disease, because the development of herd immunity was very slow. In addition, the unvaccinated adult animals remain unprotected and the infection can spread [30].

Vaccination of all animals (young and adults) in a flock or region is an alternative approach for the control of brucellosis in small ruminants. This, mass immunization is indicated where the prevalence of infected animals is high. Mass vaccination of a flock helps to rapidly establish a relatively immune stock, and reduces the level of abortions and excretes of thus reducing contamination of the environment

and disease transmission [30]. However, this strategy has the limitation that pregnant animals cannot be vaccinated because the vaccine is not innocuous enough for pregnant animals, and the efficacy of the strategy depends on the continuous availability of the vaccine [31]. Provided that the prevalence of disease is moderate, financial resources are available, and a well-functioning surveillance by the veterinary service is in place, vaccination of young animals can be combined with a test and slaughter policy in a long term action to control brucellosis in small ruminants [31].

7.4.2. Test and Slaughter

It is usually accepted that a program of eliminating brucellosis by test and-slaughter policy is justified on economic grounds only when the prevalence of infected animals in an area is about % or less [29]. For the implementation of such a program it is essential that the flocks are under strict surveillance and movement control. Animals must be individually identified and an efficient and well organized veterinary service for surveillance and laboratory testing must be in place [7, 29].

The flock size as well as the prevalence of brucellosis is the most important factors of this strategy which has been shown to be ineffective and unreliable when attempted in large flocks with a high prevalence of brucellosis [30]. Before embarking on the implementation of such a strategy it is necessary to ensure that the epidemiological situation is favorable, the necessary facilities and financial resources are available, a pool of healthy replacement animals is available and that the resources exist for continuing surveillance for a control and eradication plan based on test and slaughter strategy can be either voluntary or compulsory. Voluntary schemes, which apply to individual flocks, may be useful in the early stages of the campaign but may need to be supported by adequate incentives such as a bonus on the sale of milk from brucellosis-free herds or per capita payments. Compulsory eradication is required in the final stages but is often advisable from the start [31].

8. Conclusion and Recommendations

Poor husbandry practice, low productive potential of local breeds of small ruminants, and various parasitic, viral and bacterial diseases have made the outcome obtained from small ruminants in Ethiopia below expectation. Brucellosis of small ruminants is the most economically important disease. It is caused by *B. abortus*, *B. melitensis* and *B. ovis*. Among them the most virulent, which cause mass abortion in small ruminants during the first outbreak of the disease in the flock is *B. melitensis*. Although there are some studies which showed brucellosis in small ruminants is prevalent, information on the occurrence of a disease in different agro ecological zones of the country; the species of *Brucella* which is prevalent in small ruminants is absent. To solve the above problems, accurate diagnostic methods for brucellosis in small ruminants must be used but may be very difficult in some cases. Although there are several methods to diagnose

brucellosis in small ruminants, the only finite diagnosis is the 'gold standard', which is the recovery of the causative agent from the host specificity.

In view of this conclusion, the following points are recommended as they are very important to design strategy of control and eradication of the disease.

- (i) Veterinary laboratories should be well – equipped with skilled man power and facilities to teach students and for diagnosis of brucellosis of small ruminants.
- (ii) Modern molecular techniques of *Brucella* species identification should be incorporated in to veterinary laboratories for proper identification of the agents.
- (iii) There should be a strategy at national level to regulate the control mechanism of brucellosis in small ruminants.
- (iv) The government, Public health officers and Veterinarians have to work together to reduce economic and zoonotic impact of brucellosis.

List of Abbreviations

B	Brucella
CFSPH	center for food security and public health
CFT	complement fixation test
WHO	world health organization
ELISA	enzyme linked immuno sorbent assay
MRT	milk ring test
PAHO	Pan American Health Organization
RBPT	Rose Bengal Plate Test

Acknowledgements

First and for most, praise and gratitude is due my god for his benevolence favor on me in works of my life. Next to this, thanks to my Advisor Dr. Yosef Deneke for his intellectual guidance, provision of comment and experience sharing. I would also like to say thanks curse coordinator Dr. Moa Melaku, In addition, I would like to express my heartfelt thanks to my families for day-to-day support and encouragement. The last but not least, I thanks my friends for their experience sharing.

References

- [1] Alemu, Y. (1995): Small ruminants production in Ethiopia. *Worl. Sma. Rumin. Ani. Sci. J.*, 51: 197–201.
- [2] Glenn J. Songer and Karen W. Post, 2005. Veterinary Microbiology: Bacterial and Fungal agents of animal diseases; p-200-203.
- [3] Quinn P. J., M. E. Carter, B. K. Markey and G. R. Carter, (1994). Clinical Veterinary Microbiology; p-261.
- [4] Grimont F, Verger JM, Cornelis P, et al. Molecular typing of *Brucella* with cloned DNA probes. *Res Microbiol.* 1992; 143: 55–65.
- [5] Marin C. M., Moreno E., Moriyon I., Dazi R. & Blasco J. M. (1999) Performance of competitive and indirect enzyme-linked immunosorbent assays, gel immunoprecipitation with native hapten polysaccharide, and standard serological tests in diagnosis sheep brucellosis. *Clin. Diagn. Lab. Immunol.*, 6, 269–272.
- [6] Alton G. G., Jones L. M., Angus, R. D. & Verger J. M. (1988). Techniques for the Brucellosis Laboratory. INRA, Paris, France., Macmillan a. (1990). Conventional serological tests. In: Animal Brucellosis, Nielsen K. H. & Duncan J. R., eds. CRC Press, Boca Raton, Florida, USA, 153–197).
- [7] Dwight C. Hirsh and Yaun Chung Zee, (1999). Veterinary Microbiology; pp-197, and 201. Emmerzaal A, de Wit JJ, Dijkstra T, Bakker D, van Zijderveld FG. The Dutch *Brucellaabortus* monitoring programme for cattle: the impact of false-positive serological reactions and comparison of serological tests. *Vet Q.* 2002; 24: 40–6.
- [8] Pan American Health Organization (PAHO) 2001, Zoonoses and communicable diseases common to man and animals. 3rd Edition; V-1; Washington D. C., USA. P 53-55.
- [9] GrilloMJ., Barberan M., Blasco JM. (1997): Transmission of *Brucellamelitensis* from sheep to lambs. *Vet Rec*; 140: 602–5.
- [10] Alton G. G., Jones L. M., Angus R. D., Verger J. M. Techniques for the brucellosis laboratory Iran, Paris. 1988, p. 190.
- [11] Robles C. A, Uzal F. A., and Olacchea F. V. (1998): Epidemiological observations in a Corriedale flock affected by *B. ovis*. *Vet. Res. Commun.*, 22: 435-43.
- [12] Buchanan TM, Hendricks SL, Patton CM, Feldman RA. Brucellosis in the United States, 1960–1972: an abattoir-associated disease. Part III. Epidemiology and evidence for acquired immunity. *Medicine* (Baltimore). 1974; 53: 427–439.
- [13] Boschirolu ML, Ouahrani-Bettache S, Foulongne V, et al. Type IV secretion and *Brucellavirulence*. *Vet Microbiol.* 2002; 90: 341–348.
- [14] Young EJ, Borchert M, Kretzer FL, Musher DM. Phagocytosis and killing of *Brucella* by human polymorphonuclear leukocytes. *J Infect Dis.* 1985; 151: 682–690, (Corbeil LB, Blau K, Inzana TJ, et al. Killing of *Brucellaabortus* by bovine serum. *Infect Immun.* 1988; 56: 3251–3261.
- [15] Anderson TD, Cheville NF, Meador VP. Pathogenesis of placentitis in the goat inoculated with *Brucellaabortus*. II. Ultrastructural studies. *Vet Pathol.* 1986; 23: 227–239.
- [16] Anderson TD, Cheville NF. Ultrastructural morphometric analysis of *Brucellaabortus*-infected trophoblasts in experimental placentitis: bacterial replication occurs in rough endoplasmic reticulum. *Am J Pathol.* 1987; 124: 226–237.
- [17] Fensterbank R. (1987): Some aspects of experimental bovine brucellosis. *Ann. Rech. Vet.* 18: 421- 428.
- [18] Greiner M, Verloo D, de Massis F. Meta-analytical equivalence studies on diagnostic tests for bovine brucellosis allowing assessment of a test against a group of comparative tests. *Prev Vet Med.* 2009; 92: 373–81. doi: 10.1016/j.prevetmed.2009.07.014.
- [19] OIE Terrestrial Manual 2009 – Ovine epididymitis (*Brucellaovis*). http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.09_ovine_epid.pdf. Accessed 11 de fev. 2012. 2009b.

- [20] Bercovich, Z., Goler, L., Baysal, T., SchreuderBec, Zijderveld and Fgvan, 1998. Evaluation of the current used diagnostic procedures for detection of *Brucellamelitensis* in sheep. *Small Ruminant Research*, 31: 1-6; 27.
- [21] Corbel M. J. (2006): *Brucellosis in humans and animals*. The World Health Organization, in Collaboration with the Food and Agriculture Organization of the United Nations and the World Organization for Animal Health Geneva: WHO Press.
- [22] World Health Organization (2006): *The control of neglected zoonotic diseases: a route to poverty alleviation: report of a joint WHO/DFID-AHP meeting, 20 and 21 September 2005*. Geneva: WHO, with the participation of FAO and OIE.
- [23] Food and Agriculture Organization of the United Nations (2010): *B. melitensis in Eurasia and the Middle East*. FAO Animal Production and Health Proceedings.
- [24] Ashenafi F., Teshale S., Ejeta G., Fikru R., and Laikemariam Y. (2007): Distribution of brucellosis among small ruminants in the Pastoral region of Afar, Eastern E thiopia. *Sci. and Tech. Rev. World Organ. Ani. Health*, 26: 731–739.
- [25] Teshale T., Tadele T., Getachew T., Belay B., and Birhanu H. (2013): Sero-prevalence and risk factors study of brucellosis in small ruminants in Southern Zone of Tigray Region, Northern Ethiopia *Trop. Anim. Health. Prod*45: 1809-1815.
- [26] Nicoletti P. (1993): The Eradication of Brucellosis in Ann. *Saudi. Med. J*, 14: 4: 288-292.
- [27] Kolar, J., 1984. *Diagnosis and Control of Brucellosis in Small Ruminants*. *Preventive Veterinary Medicine* 2: 215-225.
- [28] World Health Organization (1998): *Human and Animal Brucellosis*. Report of a WHO workshop. Damascus, Syrian Arab Republic, Pp. 4-5.