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Bovine Brucellosis: Review Article

[1]*Abeje Abera and* [2]*Yitbarek Habtamu*

[1]Asela Regional Veterinary Laboratory, Oromia, Ethiopia
[2]National Institute for Control and Eradication of Tsetse Fly and Trypanosomosis, Addis Ababa, Ethiopia, P.O. Box: 19917

Abstract: Bovine brucellosis is a wide spread infectious disease affecting domestic and wildlife with serious economic and public health impact. The disease is primarily caused by *Brucella abortus* and occasionally by *Brucella melitensis*. Bovine brucellosis occurs worldwide, except a few countries that have been successfully eradicated. Since the first report of brucellosis in the 1970s in Ethiopia, the disease has been noted as one of important livestock diseases in the country. Most of the information in Ethiopia was based on serological and information on *Brucella* identification and specific transmission of the disease are scanty although isolation and the molecular techniques are considered as the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate. The commonly used serological tests in Ethiopia include Rose Bengal Plate test, Complement Fixation Test and Enzyme-Linked Immunosorbent Assay. The disease causes economic loses which arises from abortion in animals, culling of infected animal, hindering animal export trades of a country, time and costs allotted for research programs. Brucellosis in animals may be controlled by the strict enforcement of a set of measures including testing and slaughtering, vaccination, sanitation and movement control. Therefore, this review will focus on the distribution, risk factors, economic and zoonotic significance and different strategies for the control and prevention of bovine brucellosis.

Key words: Bovine Brucellosis · Cattle · Epidemiology · Diagnosis · Ethiopia

INTRODUCTION

Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals worldwide. It is considered as one of the most widespread zoonoses in the world [1]. It is estimated that brucellosis infection rates can reach higher than 10% in human populations in some developing countries [2]. In sub-Saharan Africa the epidemiology of brucellosis and its appropriate preventive measures in livestock and humans are insufficiently studied [3]. Generally, brucellosis can cause major loss of productivity through abortion, stillbirth, low herd fertility and low milk production [4].

The disease mainly affects farm laborers, slaughter-house workers, butchers, veterinarians [5]. Humans are infected by brucellosis as a result of consuming unpasteurized milk, meat and animal byproducts, from infected animals [6]. The presence of the disease in wildlife is the potential source of brucellosis for continuous transfer to domestic animals [7]. Sporadic cases may be reported in travelers and immigrants in *B. abortus*-free countries. It can be used as biological weapons and is considered a Class B potential warfare agent [8]. *Brucella* pathogens such as *B. abortus*, *B. melitensis* and *B. suis* have been identified as category B bioterrorism agents [9].

Disease transmission can occur most commonly via the digestive tract and the usual source of the organism being an infected placenta or aborted fetus [10]. Since the diagnosis of brucellosis suspected specimens is based on culture isolation and phenotypic characterization, it requires biosafety level 3 [11] protocol as it is high risk laboratory acquired infection. As a result molecular methods have been explored in order to overcome these difficulties; in addition the polymerase chain reaction based assays have shown a higher sensitivity with respect to the standard microbiological assay for the diagnosis of this disease [12].

Corresponding Author: Yitbarek Habtamu, National Institute for Control and Eradication of Tsetse Fly and Trypanosomosis, Addis Ababa, Ethiopia, P.O. Box: 19917.

There is no documented information on how and when bovine brucellosis was introduced and established to Ethiopia. In the country, there are some works on seroprevalence study of bovine brucellosis in animals and humans which has been reported from different localities of agro-ecology and production systems. So far, there has been only one recorded attempt to identify *Brucella* species in the country [13]. Accordingly the epidemiology of the infection and proportion of their natural hosts was not studied exhaustively [14, 15]. Therefore, the main objective was to review the general epidemiology, diagnosis and control of bovine brucellosis and its economic impact on production and public health

Epidemiology of Brucellosis

Etiology: Brucellosis in cattle and other Bovinae is primarily caused by *Brucella abortus*; but to a much lesser extent by *B. melitensis*, where cattle are kept together with infected goat or sheep. Brucella species is a Gram-negative coccobacillus or short rod in the family Brucellaceae (class Alpha proteobacteria). Eight *B. abortus* biovars (1-7, 9), are currently recognized. Other species of *Brucella* that may be found in cattle include *B. melitensis*, which can be important in cattle in some countries, *B.suis* and *B. canis* [16].

Occurrence

Global Distribution and Economic Impact of Bovine Brucellosis: There has been a report of brucellosis in 86 different countries worldwide, as a result the disease is distributed worldwide and it is endemic in the Mediterranean Countries of Europe, Africa, Near East countries, India, Central Asia, Mexico, Central and South America. Eradication programs in a number of European nations, the United Kingdom, Denmark, Finland, Netherlands, Canada, Australia, New Zealand, Cyprus, Norway, Sweden, Japan, Australia and Israel have eliminated bovine brucellosis from domesticated animals. [11, 17]. Brucellosis remains endemic as a result of expansion of livestock herds and flocks, with associated uncontrolled movements; lack of veterinary support services, vaccines; and husbandry practices [3]. In addition to its effect on economic loss, it is also associated with high morbidity, both for humans and animals in developing countries [18-20].

Bovine Brucellosis in Sub Saharan Africa (SSA): Bovine brucellosis is among the most widely distributed zoonoses of economic importance in sub-Saharan Africa, however the evidence obtained throughout the years showed that there is under reporting and inadequate epidemiologically valid data [3].

Even though, serological evidence of brucellosis is abundant throughout SSA, there is wide range in values, it is scattered in time and space and, in addition, the figures reported had to be interpreted with caution because of uncertainties in test implementation and validation [3].

Both classical biotyping and molecular studies show that *B. abortus* and *B. melitensis* in African countries of the Mediterranean coast are closely related to other strains in the Mediterranean basin [21]. By molecular analyses, the recent strains isolated in SSA, so far represent a genotype (*B. abortus* biovar 3a) different from (*B. abortus* biovar 3b) isolated in Europe and Latin America [22].

Status of Brucellosis in Ethiopia: There is no documented information on how and when bovine brucellosis was introduced and established in Ethiopia. However, several serological surveys have showed that higher sero-prevalence reports were 11.2% East Showa Zone [23] and 7.7% in Tigray region [24]. Furthermore sero-epidemiological study of bovine brucellosis was conducted in three separate agro ecological areas of central Oromia and the seroprevalence was 4.2% in the lowlands, 1.0% in the midlands and 3.4% in the highlands [25]. Relatively low individual animal sero-prevalence in intensive farms was recorded in different part of the country, where Edao [26] reported a prevalence of 0.06% in Addis Ababa dairy farms and Tadesse [27], observed a prevalence of 0.14% in north Gondar zone, Tadele [28] reported 0.77% in southwestern Ethiopia and Asmare [29] documented 2.46% in Sidama zone of southern Ethiopia. According to Asmare [30], *Brucella* seroprevalence in dairy cattle revealed that highest prevalence in central Ethiopia followed by southern part whereas lowest prevalence was recorded in western part of the country [31, 32].

Host Range and Brucella Diversity: The principal strain that infects cattle is *B. abortus*; cattle can also become transiently infected by *B. suis* and more commonly by *B. melitensis* when they share pasture or facilities with infected pigs, goats and sheep *B. melitensis* and *B. suis* can be transmitted by cow's milk and cause a serious public health threat [53-55]. The main etiologic agent of brucellosis in goats and sheep is *B. melitensis*.

Table 1: Sero-prevalence of bovine brucellosis from different locations of Ethiopia

	Test type		
	RBPT	CFT	Reference
1. Jimma zone, S/W Ethiopia	3.3	3.1	[33]
2. Bahr Dar	4.63	-	[34]
3. Tigray (Tabias)	3.33	3.19	[35]
4. Tigray	4.9	-	[36]
5. Wuchale-Jida district	12.5	11.0	[37]
6. East Showa Zone	11.2	-	[23]
7. Central Oromia	-	2.9	[25]
8. Hwassa	3.9	-	[38]
9. Arsi-Negele	-	2.6	[39]
10. Sidamo zone	-	1.66	[40]
11. Addis Ababa dairy farms	2.5%	1.5%	[41]
12. Selected Districts of Arsi Zone	0.5	0.5	[42]
13. Jigjiga zone	1.84	1.38	[43]
14. Southern & Eastern Ethiopia	-	3.5	[44]
15. Western Tigray	-	6.1	[36]
16. Guto-Gida district, East Wollega Zone	2.96	1.97	[32]
17. Representing Ethiopia	-	1.9	[40]
18. SE. Somali and Oromia	-	0.9	[45]
19. Benishangul Gumuz	1.2	1	[46]
20. Debre-Zeit, Central Ethiopia	3.3	2	[47]
21. Adami Tulu	4.5	4.3	[48]
22. Debrebirhan and Ambo Towns	0.7	0.2	[49]
23. Bishoftu and Asela	2.28	-	[31]
24. In and around Alage district	2.4	2.4 (cELISA)	[50]
25. North West Gondar	5.4	4.9	[51]
26. North Shewa	0.78	-	[52]
27. Addis Ababa dairy farms	2.77	0.06	[26]

In certain countries like Brazil where there is no *B. melitensis*, goats got infected with *B. abortus* [56]. Camels can be infected by *B. abortus* and *B. melitensis* when they are pastured together with infected sheep, goats and cattle. Milk from infected camels represents a major source of infection that is underestimated in the Middle East [57]. The main etiologic agent for dog brucellosis is *B. canis*, but sporadic cases of brucellosis in dogs caused by *B. abortus, B. suis* and *B. melitensis* have been reported [55].

Transmission: As a herd problem, it is primarily spread by contact and ingestion of contaminated material [12, 58], while spread between herds is facilitated by introduction of asymptomatic animals [59]. The primary routes of infection are through the mucous membranes of the conjunctiva, oral and nasal surfaces [60] and supposedly through vertical transmission by infecting new-born calves and lambs in the uterus or colostrums [61]. Humans get infected by direct or indirect contact with infected animals or by ingestion of their products or by-products [62-64]. While pasteurizing milk is an effective means to kill *Brucella* and prevent infection in humans, this precaution is not routinely practiced in some resource limited communities because of long- standing cultural practices and a generalized lack of understanding by the public about the dangers of consuming raw milk [65]. Human-to-human transmission can occur transplacentally, via breastfeeding and rarely through sexual intercourse, organ transplantation and blood transfusions have been observed in rare cases [66].

Risk Factors: Susceptibility of cattle to *Brucella abortus* infection is influenced by: management factors such as movement and congregation of animals for access to pastures, water, or marketing; artificial insemination [67-70], herd sizes and population density; animal factors such as age, sex, reproductive status; and biological factors such as herd immunity [71]. Infection occurs in cattle of all ages but persists commonly in sexually mature animals [58].

Immune Response to Brucella Infection: Protective immunity to the host is conferred by T-cell mediated macrophage activation by the antigenic protein of *Brucella* and the production of corresponding antibody

along with other elements of immune response such as tumor necrosis factor (TNF), interferons and complement [72-75]. Following infection, the immunoglobulin M (IgM) titer increases initially followed by the immunoglobulin G (IgG) titer [76, 77]. Thus, the appearance of IgM indicates an early immune response against brucellosis and IgG correspondingly indicates chronic infection or relapse [78, 79].

DIAGNOSIS

Diagnostic Procedures: The diagnosis of brucellosis is based on serological, bacteriological, allergic skin reaction and molecular methods [80]. The most important confirmatory method of *Brucella* infection is bacteriological diagnosis since its specificity is much higher than that of other diagnostic methods and it is used as a gold standard diagnostic method [81-83]. Sample transport has to be rapid and cultural growth should start within 1-2 hours after sample taking. For longer transport times, clinical samples should be cooled to 2-8°C, tissue samples have to stay moist during transport [84-88].

Direct Methods for Diagnosis of Brucellosis: Isolation of the organism is considered the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant under an epidemiological point of view [85, 89]. However, in spite of its high specificity, culture of *Brucella* spp. is challenging. *Brucella* species are a fastidious bacterium and requires rich media for primary cultures [10, 82, 90]. The smooth lipopolysaccharides (LPS) that cover the bacterium and proteins involved in signaling, gene regulation and transmembrane transportation are among the factors suspected to be involved in the virulence of *Brucella* [91-93].

Importantly, brucellosis is one of the most common accidental laboratory infections; particularly in research laboratories [94-98]. Samples for *Brucella* isolation from cattle include fetal membranes, particularly the placental cotyledons where the number of organisms tends to be very high. In addition, fetal organs such as the lungs, bronchial lymph nodes, spleen and liver, as well as fetal gastric contents, milk, vaginal secretions and semen are samples of choice for isolation [96, 99, 100]. *Brucella* colonies are elevated, transparent and convex, with intact borders, smooth and a brilliant surface.

Identification of *Brucella* strains is done using standard classification tests, including Gram stain, a modified Ziehl-Neelsen (ZN) stain, growth characteristics, oxidase activity, urease activity, H_2S production (four days), dye tolerance such as basic fuchsine (1: 50000 and 1: 100000) and thionin (1:25000, 1:50000 and 1:100000) and seroagglutination [101].

Molecular Technique: The use of the Polymerase Chain Reaction(PCR) to identify *Brucella* DNA at genus, species and even biovar levels has becoming extended to improve diagnostic tests and a diversity of methods have been developed. It is rapid, safe and cost effective, the only real problems being some uncertainties regarding specificity [100]. In addition to the commonly used PCR assays, a new Multiplex-PCR assay was developed that specifically identified *B. neotomae*, *B. pinnipedialis*, *B. ceti* and *B. microti*. Furthermore, it differentiated *B. abortus* biovars 1, 2, 4 from biovars 3, 5, 6, 9, as well as between *B. suis* biovar 1, biovars 3, 4 and biovars 2 and 5 [102-104].

Indirect Methods for Diagnosis of Brucellosis

Rose Bengal Plate Test (RBPT): It is a spot agglutination technique. It does not need special laboratory facilities and is simple and easy to perform. It is used to screen sera for *Brucella* antibodies. The test detects specific antibodies of the IgM and IgG type. Although the low PH (3.6) of the antigen enhances the specificity of the test and temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the test [106].

The drawbacks of RBT include: low sensitivity particularly in chronic cases, relatively low specificity in endemic areas and prozones make strongly positive sera appear negative in RBT [107]. The overall sensitivity is 92.9%, so the use of RBT individuals exposed to brucellosis and those having history of *Brucella* infection. Rose Bengal plate test (RBPT) is an agglutination test that is based on reactivity of antibodies against smooth lipopolysaccharide (LPS). As sensitivity is high, false negative results are rarely encountered. To increase specificity, the test may be applied to a serial dilution (1:2 through 1:64) of the serum samples [108, 109].

Complement Fixation Test (CFT): Due to its high accuracy, complement fixation is used as confirmatory test for *B. abortus, B. melitensis* and *B. ovis* infections and it is the reference test recommended by the OIE for international transit of animals [110, 111]. However, this method has some disadvantages such as high cost, complexity for implementation and requirement for special equipment and trained laboratory personnel [112]. Sensitivity of complement fixation ranges from 77.1 to 100% and its specificity from 65 to 100% [11, 113].

Enzyme Linked Immunosorbent Assay (ELISA): It is the test of choice for complicated, local or chronic cases particularly when other tests are negative while the case is under high clinical suspicion. It can reveal total and individual specific immunoglobulins (IgG, IgA and IgM) within 4-6 hours with high sensitivity and specificity. In addition to the detection of immunoglobulin classes, ELISA can also detect *Brucella*-specific IgG subclasses and other *Brucella* immunoglobulins such as IgE [114-116].

Milk Ring Test (MRT): The test 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 [117, 118].

2-Mercaptoethanol (2-MET): The 2-MET is an adaption of the SAT titer. There are two forms of this test, which uses either 2-mercaptoethanol or Dithiothreitol [119]. Dithiothreitol is preferable because of the toxicity of 2-mercaptoethanol. The test measures mainly IgG, because the disulphide bridge of IgM is being reduced to monometric molecules and, therefore, unable to agglutinate [120].

Fluorescence Polarization Assay (FPA): The Fluorescence Polarization Assay (FPA) is based on the fact that, when polarized light excites fluorescent molecules, they will emit polarized light. The FPA can distinguish vaccinal antibody in most vaccinated animals and it can as well eliminate reactivity by some cross-reacting antibodies [121]. Sensitivity of the fluorescence polarization assay varies from 87.5 and 100% and specificity from 84 to 100% [118], which is similar to the levels obtained with c-ELISA [122].

Agar Gel Immunodiffusion (AGID) Test: The Agar Gel Immunodiffusion (AGID) test is based on the precipitation of the antigen-antibody complex. This method is often used for the diagnosis of *B. ovis* infection. This test has a low cost, it is easily performed and it has sensitivity levels that are comparable to complement fixation. Therefore, it is highly advisable to perform complementary diagnostic techniques such as PCR [123]. Sensitivity of the agar gel Immunodiffusion test varies from 50 to 92.7% and the specificity from 94.3 and 100% [124, 125].

Table 2: Screening and confirmatory tests used in the serological diagnosis of *Brucella* spp. infection

Species	Screening tests	Confirmatory test	Reference
B. abortus	BPAT, MRT	2ME, CFT, cELISA	[126]
B. melitensis	BPAT	BPAT, CFT	[127]
B. suis	BPAT	2ME, CFT, AGIT, cELISA	[128]
B. canis	-	2ME, AGIT, ELISAi	[129]
B. ovis	-	CFT, AGIT, i-ELISA	[125, 130]

Coombs Test: This is the most suitable and sensitive test for confirmation of relapsing patients with persistent disease [131]. It is an extension of the SAT test i.e., if the SAT test yields negative results due to the presence of blocking antibodies, Coombs test may be used instead. Coombs test is used for detection of incomplete, blocking or non-agglutinating IgG. It is good for complicated and chronic cases but misses about 7% of cases compared with ELISA [129, 132].

Dipstick Assay: The IgM dipstick assay is one of the tests that have been adapted to detect IgM antibodies to the smooth LPS. The assay has shown high sensitivity for patients with disease lasting less than 3 months [133, 134].

Lateral Flow Assay: An immunochromatographic *Brucella* IgM/IgG lateral flow assay is a simplified version of the ELISA test and has a great potential as a rapid point-of-care assay. The test has high sensitivity and specificity for *Brucella* IgM and IgG. It uses a drop of blood obtained by finger prick. It can be done as a bedside procedure. So it is a rapid and a simple diagnostic test that is also easy to interpret [131].

Rapid Slide Agglutination Test (RSAT): The rapid slide agglutination assay test (RSAT) could be a suitable screening test for the diagnosis of human brucellosis and a supplementary technique, such as ELISA, performed on all positive RSAT samples that were negative by *B. abortus* antigen could ensure diagnostic specificity and confirm the diagnosis [135, 136].

Brucellin Allergic Skin Test: The injection of brucellergene, a protein extract of a rough strain of *Brucella* species is followed by a local inflammatory response in a sensitized animal.

Treatment: In underdeveloped countries, treatment of cattle is not a common practice; however, the infected animals are isolated, culled or slaughtered to prevent the spreading of infection to other herd and at substantial veterinary costs. Generally, treatment of infected livestock

is not attempted because of the high rates of treatment failure, cost, [136, 137] and potential problems of residues to public safety when high doses of antimicrobials are used as chemotherapy. Man can be treated with a combination therapy of Doxycycline and Rifampicin antimicrobials, however, relapses may occur [62]. The World Health Organization recommends that acute brucellosis cases be treated with oral doxycycline and rifampicin (600 mg for six weeks) [138, 139].

Prevention and Control: Brucellosis in animals may be controlled by the strict enforcement of a set of measures including testing and slaughtering, vaccination, sanitation and movement control [18].

Several countries have been declared brucellosis free because of continuous efforts and implementation of strategic control measures for eradication. Reducing or eliminating the source or reservoir of infection by quarantine, destruction of reservoir, early detection of disease and environmental control [140]. New Zealand is free from brucellosis and the methods used for eradication exemplify a range of disease control strategies such as stamping out affected herds, compulsory treatment, vaccination and test and removal [141].

Impact of Brucellosis on Production and Public Health: Brucellosis is a 'multiple burdens' disease with economic impacts attributable to human, livestock and wildlife disease [142]. Losses in animal production due to brucellosis can be primarily because of the decreased milk production by aborting dairy animals; the common sequel of infertility increases the period between lactation and in an infected herd the average inter calving period may be prolonged by several months [58]. The modeling estimates predicted that eradicating the disease from this region would generate between USD $0.497– USD $1 billion in additional income potential for stakeholders on an annual basis [143]. Furthermore, a study conducted in India in 2015 attributed a median loss of USD $3.4 billion of revenue to the livestock sector from the production losses, reduction in fecundity and premature deaths of animals infected with brucellosis [144, 145]. Furthermore, forgone revenue related to brucellosis includes trade restrictions from areas endemic with *B. melitensis, B. abortus* and *B. suis*.

International travel and the importation of different dairy products into *Brucella* free regions contribute to the ever-increasing concern over human brucellosis. All ages of human beings are susceptible and even congenital cases have been recorded [146, 147]. In human the disease cost of treatment and absenteeism from work brings many economical impacts [148].

CONCLUSION

Brucellosis stands first in the list of zoonotic bacterial diseases. The gold standard for diagnosis of brucellosis is the isolation and phenotypic characterization of the organism, but this is a laborious and slow technique that requires well trained personnel and biosafety level 3 laboratories. Therefore, molecular methods have been increasingly used for a definitive diagnosis. Brucellosis can be prevented in humans by controlling, or better, eliminating the disease in the animal population, avoiding consumption of raw milk, raw milk products and adopting hygienic practices.

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