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A Review on Brucellosis: Epidemiology, Risk Factors, Diagnosis and Management Strategies

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Abstract: Brucellosis is a zoonotic disease imposing significant impacts on livestock production and public health worldwide. This review aimed to throught light on the Brucella epidemiology, risk factors and diagnosis and management strategies. The disease is caused by diverse Brucella species of which *Brucella aborts*, *B. melitensis* and *B. suis* are highly pathogenic. Brucella can be transmitted via horizontal or vertical routes. Brucella organisms are found in higher concentration in the uterus of pregnant animals. The aborted fetuses, placental membranes and uterine discharges act as main source of infection. Laboratory workers handling Brucella cultures are at high risk of acquiring brucellosis trough accidents aerosolization and inadequate laboratory procedures. Generally, smooth lipopolysaccharides have a role in cell entry and immune evasion of the infected cell. It also alters the capacity of the infected cell to present foreign antigens, hence, prevents the immune system attack for the infected cell. The clinical signs, manifestations and multiple complications in brucellosis in different animal species are firstly related to the reproductive tract. In human, the main presentations are acute febrile illness, with or without signs of localization and chronic infection. The use of molecular biology as a diagnostic tool is advancing and will soon be at the point of replacing actual bacterial isolation rather than other diagnostic techniques. The distribution of brucellosis in different geographies is highly dynamic, with emergence of new areas of infection and re-emergence of infection in areas where infection existed earlier. In Ethiopia studies in many parts by different persons on the prevalence of brucellosis ranges from 0.5 % - 11.2%. Thus, awareness creation for the society about public and economic significances of the disease is essential in reducing burden of the disease as well as One Health approach can aid in control of this disease, both in animals and man.

Key words: Animal · Brucellosis · Human · Economic Significance · Zoonosis

INTRODUCTION

Brucellosis is a highly contagious zoonotic disease and a cause of significant reproductive losses in livestock. Brucellosis is described as enzootic and is common in low- and middle-income countries [1]. The disease caused by the genus Brucella and within the genus there are six species namely *B. aborts*, *B. mellitensis*, *B. suis*, *B. ovies*, *B. neotomae* and *B. microti*. Brucella organisms are transmitted through contaminated and unpasteurized milk, milk products or by direct contact with infected animals or animal carcasses [2]. Animal brucellosis causes direct socio-economic effects in communities who depend on animal production for their livelihood [3]. Losses in animals are attributed to direct effects on their offspring due to abortion, stillbirth and infertility whereas indirect losses are due to reduction in milk yields and human suffering resulting from the disease [4, 5].

In low- and middle-income countries LMICs, the prevalence of animal and human brucellosis is generally unknown due to a myriad of challenges with diagnostics, reporting and weak to non-existent surveillance systems, especially in malaria endemic areas with variations based on the pastoral systems [6]. Although prevalence is high and variable in many countries, surveillance for the disease is generally poor factors assumed to be responsible for variation in prevalence include purchase of infected cattle from the market for replacement or upgrading, nature of animal production, sharing of bulls,

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use of open-range grazing, demographic factors, regulatory issues and climate and wildlife interaction [7]. Moreover, one major factor contributing to the spread of the disease is the free movement of animals practiced by the livestock keepers. Despite under-reporting and inadequate epidemiologically valid data, the evidence obtained throughout the years illustrate that brucellosis is a widespread problem in Africa, a continent where several Sub-Saharan countries are estimated to bear a high burden of neglected zoonotic diseases [8]. In terms of socio-economic effects, it has been documented that most quantifiable expressions of Brucella are linked to reproduction [6, 9]. For example, infected male animals were prone to infertility and reduced reproductive performance. Female animals, on the other hand, suffered from abortion, stillbirth and early death of offspring when the uterus gets infected. In addition to spreading the infection to human, animal brucellosis impacts livestock productivity, which can have adverse socio-economic and indirect health consequences on human, especially helpless livestock-keeping populations in resource-limited surroundings that depend on livestock for food security and income [10].

The impacts of brucellosis in livestock include abortion and death as well as decreased milk and meat production and reduced reproductive efficiency [11]. Generally, the costs associated with the treatment in animals attributed to diseases such as brucellosis is remarkably high [12]. As the disease is hardly remarkable in its chronic stage and despite the losses and yield decrease, its causes often goes unnoticed. Its negative effect on cost-effectiveness of livestock production is extremely undervalued particularly in tropical areas in wide-ranging management system [13]. Brucellosis illness to the herds reduces livestock production and reproduction performance evident by frequent episodes of abortion especially during the last trimester, retention of placenta, metritis, birth of weak calves, infertility in bulls and cows and 20% reduction in milk production from infected cows [14-16]. Therefore, this review aimed to spot the light on the brucella epidemiology, risk factors, diagnosis and management strategies.

Etiology: Brucella is a highly contagious zoonotic disease and come under a–2 subdivision of Proteobacteria [17]. A total of six classical and seven novel Brucella species have been recognized from a wide spectrum of susceptible hosts. Species affecting terrestrial animals are seven in number including *B. aborts, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae* and *B. microti* [18].

They are Gram-negative, aerobic, facultative intracellular rods or coccobacilli, which lack capsules, endospores or native plasmids. The bacterium has a diameter of 0.5–0.7mm and has 0.6–1.5 mm length, partial acid fast with oxidase, catalase and nitrate reductase and urease activity. The Brucellae are able to survive freezing and thawing, but are susceptible to most of the common disinfectants. The bacterium remains viable in environment for months especially in cool and wet conditions. Pasteurization can effectively kill Brucella in milk. Though they are non-motile, yet they have all the genes except the genes required to form a flagellum.

The two other species*, B. ceti* and *B. pinnipedialis* affect marine mammals [19]. *B. papionis* isolated from baboons and B. vulpis from red foxes were also added to the list of genus Brucella [20]. Moreover, seven biovars have been recognized for *B. aborts*, three for *B. melitensis* and five for *B. suis*. Rest of the species has not been characterized into biovars. The Brucella nomenclature is based on the principal host species. Reports also document the isolation of 36 atypical *Brucella spp.* from frogs [20, 21].

As the list of species increases, it is essential to identify better prevention measures to control the spread of disease to man. The genomes of all Brucella species are having similar size and genome atlas), with average genome size of approximately 3.29 Mb consisting of two circular chromosomes [22].

Chromosome I is approximately 2.11 Mb and chromosome II is about 1.18 Mb. The G þ C content of chromosome I is 57.2% and chromosome II is 57.3% [23]. The classic virulence genes for plasmids, capsules, pili or exotoxins are absent in *Brucella* species. A draft genome sequence of B. aborts SKN13, isolated from placenta of aborted cattle from Gujarat state of India has proved very useful in providing insight into comparative genomic analysis of Brucella strains from India [24]. *Brucella* isolates in different country have been molecularly characterized from cow milk [25].

A genomic monomorphism was found in isolates and showed significant genetic variation when compared with other B. aborts biovars from Africa and other countries of the globe. McDermott and Arimi [26] focused on genome-wide single nucleotide polymorphisms (SNP) based-genome-wide association studies for identification of the genetic determinants in Brucella species and could identify 143 species-specific SNPs in B. aborts conserved in 311 B. aborts genomes, of which as many as 141 SNPs common disinfectants. The bacterium remains viable in environment for months especially in cool and wet

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Epidemiology

Geographical Distribution: The distribution of brucellosis in different geographies is highly dynamic, with emergence of new areas of infection and re-emergence of infection in areas where infection existed earlier. New areas of prevalence of human brucellosis have emerged in Central Asia and Middle East countries where prevalence is continuously increasing [22]. This disease is prevalent throughout the world except in Canada, Australia, Cyprus, Norway, Finland, the Netherlands, Denmark, Sweden, New Zealand and United Kingdom. However, Mediterranean Europe, Central and South America, Mexico, Africa, Near East countries, Central Asia, India and Italy are having significant prevalence of brucellosis. Brucellosis is a reportable and notifiable disease in several countries; however, gross underreporting is a glaring problem [23].

A report considering 19years (1996–2014) by the World Organization for Animal Health (OIE) regarding 156 countries classified the countries into three groups based on the situation of brucellosis among animals. The three categories are: enzootic for brucellosis: countries that are infected or free of brucellosis for less than 3years time period, non enzootic for brucellosis: though brucellosis may be present, countries in this category are devoid of disease for a period of 3years and free of brucellosis: countries devoid of brucellosis throughout the study period of 19years. The disease-free status countries are situated in Europe and Oceania while high prevalence or enzootic countries are present in Central and South America, Africa and parts of Asia [24].

Brucellosis is endemic in Western Asia, India, Middle East, Southern Europe and South America [25, 26]. Study in Iran reported that B. aborts biovar 3 is the most prevalent biovar [27] Reports of low incidence of brucellosis in endemic areas could be due to either inadequate surveillance or under reporting [28]. Brucellosis is mainly caused by B. aborts biovar 1 in water buffaloes in parts of Africa, South America, Brazil, Italy, Pakistan and Egypt [29]. In Italy, cattle and water buffalos both are affected by *B. aborts* mainly in southern areas. In Egypt, brucellosis is an endemic problem [30].

Reports of B. melitensis infection in cattle are pouring which is a major threat in Kuwait, Saudi Arabia, Israel and some southern European countries [31]. Although in most countries brucellosis is a nationally notifiable disease and reportable to the local health authority, it is under reported and official numbers constitute only a fraction of true incidence of the disease [32]. New human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening [33].

Source of Infection and Mode of Transmission: Brucella can be transmitted via horizontal or vertical route [34]. Brucella organisms are found in higher concentration in the uterus of pregnant animals. The aborted fetuses, placental membranes and uterine discharges act as main source of infection. Organisms shed in the milk of infected animals may transmit the infection to the newborn. The organism may survive in the environment for months together especially in cold and moist atmosphere. The animals contract the infection by ingestion of contaminated feed and water or by contacting aborted fetuses, fetal membranes and discharges from uterus. Inhalation could also be a mode of transmission. Infected bulls may also spread infection by natural service or artificial insemination from one herd to another [35].

Risk Factors

Pathogen Risk Factor: *Brucella aborts* is a facultative intracellular organism capable of multiplication and survival within the host phagosome. The organisms are phagocytized by polymorphonuclear leucocytes in which some survive and multiply. The organism is able to survive within macrophages because; it has the ability to survive phagolysosome [36]. The bacterium possesses an inconveniently non-endotoxin lipopolysaccharide (LPS),

which confers resistance to antimicrobial attacks and modulates the host immune response. These properties make lipopolysaccharide an important virulence factor for Brucella survival and replication in the host [37].

Host Risk Factors: Susceptibility of cattle to *B.aborts* infection is influenced by the age, sex and reproductive status of the individual animal. Sexually mature pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex. Susceptibility increases as stage of gestation increases [38].

Management Risk Factors: The spread of the disease from one herd to the other and from one area to another is almost always due to the movement of an infected animal from infected herd in to a non-infected susceptible herd. A case-control study of brucellosis in Canada indicates that, herds located close to other infected herds and those herds whose owners made frequent purchase of cattle had an increased risk of acquiring brucellosis. Once infected, the time required to become free of brucellosis was increased by large herd size, active abortion and by loss housing [37].

Occupational Risk Factors: Laboratory workers handling Brucella cultures are at high risk of acquiring brucellosis trough accidents, aerosolization and inadequate laboratory procedures. In addition to this, abattoir workers, farmers and veterinarians are at high risk of acquiring the infection [36].

Pathogenesis: According to a number of studies point at the outer membrane being the main component for virulence factor of Brucella, this membrane contains LPS [39]. It possesses a peculiar non-classical LPS as compared to the classical LPS from Enterobacteria, such as Escherichia col. Generally, smooth LPS have a role in cell entry and immune evasion of the infected cell. It also alters the capacity of the infected cell to present foreign antigens, hence, prevents the immune system attack for the infected cell. LPS has three domains: lipid A, the core oligosaccharide and the O-antigen or O-side chain. The O-polysaccharide of smooth-type Brucella LPS (S-LPS) is an unbranched homopolymer of 1, 2-linked 4, 6-dideoxy-4-formamido-α-D-mannopyranosyl usually with an average chain length of 96 to 100 glycosyl subunits. The O-polysaccharide is linked to a core oligosaccharide composed of mannose, glucose, 2-amino-2, 6-dideoxy-D-glucose (quinovosamine), 2- amino-2-deoxy-D-glucose (glucosamine), 3-deoxy-Dmanno-2-octulosonic acid (KDO) and unidentified sugars [39].

The lipid A, linked to the core oligosaccharide, contains 2, 3-diamino-2, 3-dideoxy-Dglucose (diaminoglucose) as backbone, amide and ester-linked long chain saturated (C16:0 to C18:0) and hydroxylated (3-OH-C12:0 to 29-OH-C30:0) fatty acids. The hydrophobic lipid a region constitutes mostly the outer coating of the outer membrane and is responsible for many of the endotoxic properties attributed to LPS. Thermotropic phase behavior and immunochemical analysis of *B. aborts* and *B. melitensis* lipid A suggest a disaccharide backbone molecule linked in a β1 –6 configurations. Ethanolamine, neutral sugars and ester-linked acyloxyacyl fatty acids are not found and phosphate is absent or present in reduced quantities. Brucella lipid contains strongly bound outer membrane protein fragments that are not removed by conventional procedures used to release the lipid-A-associated protein of enterobacterial LPS [40].

The lipopolysaccharide coat being smooth in *B. melitensis, aborts, suis* and rough in *B. canis* can inhibit phagosomal fusion and oxidative burst activity. Phagocytes can readily kill *B. aborts* resulting in development of tissue granulomas and rarely ingest *B. melitensis* resulting in visceral micro-abscesses; thus explaining the differences in pathogenicity and clinical manifestations in human cases of brucellosis [41]. This leaves about 15 to 30% of Brucella alive which is transported into the lymphatic system and may cause systemic infection [42]. After replication in the endoplasmic reticulum, the Brucella are released with the help of hemolysins and induced cell necrosis. Development of cell mediated immunity controls Brucella infection and helps in the recovery. Some immunity to reinfection is provided by serum immunoglobulin (Ig): IgM antibodies may remain in the serum in low levels for several months, IgG declines but persistent elevation indicates chronic or relapsed infection and IgA may persist for very long intervals [43].

Clinical Signs

Clinical Signs in Animals: Various clinical signs have been described in infected animals; the main manifestation in *B. aborts* infection being reproduction failure in the form of abortion and birth of weak offspring's which remain as carrier in herd. The clinical signs, manifestations and multiple complications in brucellosis in different animal species are firstly related to the reproductive tract. The incubation period could vary from two weeks to months together. Calves could be infected at early stage but no symptoms are seen till they mature. It is manifested

by late abortions in pregnant animals, birth of weak calves, lowered fertility, retention of fetal membranes, endometritis and reduction in milk production [43].

Abortion rate may vary from 30 to 80% in susceptible herds [44]. Calves borne at full-term may die very soon after birth. Fibrinous pleuritis coupled with interstitial pneumonia also appears in newborn calves and also in aborted fetuses [45]. Male animals show clinical manifestations in the form of orchitis and epididymitis, whereas, hygroma is witnessed in chronic infections [46]. Cervical bursitis in cattle has also been reported due to brucellosis. In the seminal vesicles, the acute inflammatory phase is followed by a chronic stage with considerable fibrinoid induration. Areas of dry necrosis develop and become encapsulated by fibrinous tissue, which eventually contracts, often leaving the testicles smaller than normal. Orchitis and epididymitis are typical signs in males and hygroma is usually common during chronic infection [47].

In highly susceptible non vaccinated pregnant cow, abortion occurs after the 5th months of pregnancy in bull, orchitis and epididymitis are cardinal signs. In case of horse, it is usually associated *B. abortus* with chronic bursal enlargement of the neck and withers and abortion in mares. Brucellosis in swine has acute symptoms like abortion, infertility and birth of weak piglets, orchitis, epididymitis and arthritis. Sheep and goats have similar to that observed in other species of animals [48].

Abortion in goats occurs most frequently in the third or fourth months of pregnancy. In case of dog and cats, infertility either in male or female, abortion and still birth or weak puppies are common manifestations. Infected livestock exhibit clinical signs of great economic significance to small and large scales livestock farmers and industries. Characteristic but not specific signs of brucellosis in most animal hosts are abortion or premature births and retained placenta. Interference with fertility is usually temporary, most infected animals will abort only once and some are unaffected [30].

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 [24]. Other signs can include arthritis in cows and pigs, Splenic abscesses and small intestinal adhesions on post-mortem examination in sows , orchitis or epididymitis in the case of *B. melitensis* and *B. ovis* in sheep [25]. Mastitis and lameness in goats and oozing skin lesions in horses (fistulous withers) Additionally, it can induce a substantial decline in milk production over an animal's lifespan, often udder is permanently infected, especially in cows and goats, with continuous shedding of the organism in milk [26].

Clinical signs of brucellosis in camels appear to be very rare [31]. In addition, clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of Brucella spp. either by isolation of the bacteria or detection of their antigens or genetic material, or by demonstration of specific antibody or cell-mediated immune responses [49].

Clinical Signs in Human: In human, the main presentations are acute febrile illness, with or without signs of localization and chronic infection. Range of non-specific clinical signs may be observed including malaise, fatigue, sweats, anorexia, headache, depression, abdominal or back the main presentations are acute febrile illness, with or without signs of localization and chronic infection [49]. The fever of brucellosis may mimic that of enteric fever and an undulant fever pattern is seen in chronic infections. Fever may be absent among patients with end-stage renal disease who acquire brucellosis [49].

Mild lymphadenopathy is seen in 10 to 20% of patients; and splenomegaly or hepatomegaly in 20 to 30%. Hepatosplenic abscesses are visualized through imaging in 1.2% of cases and rare instances of splenic rupture have been reported. Bone and joint infections are common, including a high rate of vertebral osteomyelitis instances of acute or sternotomy infection, granulomatous myositis, bursitis and soft tissue or muscular abscesses. Most cases of Brucella mono-arthritis represent reactive rather than septic disease Infection of natural or prosthetic joints (24 cases reported to2016) and soft tissue. Subclinical sacroiliitis is common. Asymptomatic infection has also been reported [34].

Clinical and laboratory features vary widely. Endocarditis is well documented including isolated case reports of Brucella infection of prosthetic valves and devices such as implantable defibrillators and pacemaker leads. Rare instances of aortitis venous or arterial thrombosis, myocarditis and pericarditis have been reported [50].

Diagnosis: Specimens for culturing must be carefully collected and appropriately handled during transportation. In Bacteriological test and appropriate facilities are needed to isolate and identify all suspect *Brucella species* from abortion materials (fetal stomach contents and cotyledons), blood, milk and vaginal discharges, as well as tissues from slaughtered reactor

animals, such as supra mammary lymph nodes. The use of highly selective culture media and the development of equipment's for maceration of tissues have made isolation of Brucella more rewarding task [2, 17].

The most common basal media in use are tryptase soy, bacto tryptose, triptic soy and tryptone soya. Frequently, field samples are contaminated with other bacteria, thus, selective media should be used to avoid overgrowth by fast growing agents. The use of selective culture media is needed to increase the probability of success of bacterial culture and it is compulsory for the adequate bacteriological diagnosis of brucellosis [2]. Any basal media mentioned above with agar may be used to prepare selective media. The most widely selective media used are the kuzdas, Morse and farrell´s mediums. The kuzdas and morse use the following antibiotics and quantities per liter of basal medium, 100 mg of cycloheximide (fungistat), 25, 000 units of bacitracin (active against gram-positive bacteria) and 6, 000 units of polymyxin B (active against gram-negative bacteria [51].

Classical identification and typing of *Brucella species* in to their respective species and biovars are the work should be undertaken after culturing any suspected specimen in appropriate media. After 48-72h of incubation at 37°C, Brucella colonies are 0.5 to 1.0 mm in diameter with a convex and circular outline. Smooth strains (*B.aborts, B. melitensis and B. suis)* are transparent and pale yellow, resembling droplets of honey with a shiny surface when observed in transmitted light. Rough colonies (*B. ovis* and *B. canis*) are more-opaque with a granular surface when compared with the smooth strains of Brucella organisms. Dissociation of Brucella can be detected by the emulsification of a colony in 0.1% w/v aqueous acriflavine. Smooth colonies, *B. aborts, B. melitensis* and *B. suis* produce a yellow uniform suspension whereas rough colonies *B. ovis* and *B. canis* produce granular agglutinates. Colonial variation can be detected also by examining the plates under oblique light after staining the colonies with crystal violet. Smooth colonies appear translucent and pale yellow and rough colonies are stained with red, purple or blue with opaque and granular appearance [17].

Colonial morphology, staining, slide agglutination with anti-Brucella serum (smooth or rough), urease, catalase and oxidase tests are the basis for a culture to be identified as belonging to the genus Brucella. Once a culture has been identified as Brucella, it is important to classify the species and the biovars. This further classification of such agents should be done in well specialized or reference laboratories that have full necessary facilities and requirements for classification and identification purposes without any confusing and challenging accordingly. These tests are cumbersome and include carbon dioxide requirement (CO2), production of hydrogen sulphide (H*2*S), dye sensitivity (thionin and basic fuchsin), phage lysis, agglutination with specific antisera and in some cases, it is necessary to use the oxidative metabolic method. This latter test is time consuming and hazardous to laboratory personnel. For these reasons it should be performed only by international reference laboratories [17, 51]

Serological Tests: Serological tests can be divided broadly into two groups and these are screening tests and confirmatory tests. Some screening tests used in the field clinics or in regional laboratories, such as the Rose Bengal, buffered plate agglutination test (BPAT). Rose Bengal plate test (RBPT) has a very high sensitivity to ensure that infected animals are not missed. The milk ring test is also an excellent screening test for dairy cattle. Indirect ELISA tests are also being used to screen milk and serum. Confirmatory tests include Complement Fixation Tests (CFT), competitive ELISA, Fluorescence Polarization Assay (FPA) are very useful in distinguishing vaccinal antibody responses from those induced by field infections [52].

In RBPT, *B aborts*s99 or s1119.3 cells are stained with Rose Bengal or Brilliant Green while in BPAT the cells are stained with Crystal Violet and suspended in a buffer which when mixed with the appropriate volume of serum results in a final PH of 3.65.This PH discourages agglutination by IgM but encourages agglutination by IgG1, reducing cross reaction. Antibody resulting from *B. aborts*s 19 vaccination will react in these tests. These testes are considered as a suitable screening test for brucellosis followed by confirmatory tests like CFT [17, 52].

In Milk Ring Test, the agglutination test has been adapted to test milk for antibody to *Brucella species*. The format of this test is a little different in that hematoxylin-stained Brucella cells are added to whole milk. The reaction is allowed to take place. Immunoglobulins present in the milk will in part be attached to fat globules *via* the Fc portion of the molecule. If antibody to Brucella species is present, agglutination will take place resulting in a purple band at the top of the milk. If no antibody is present, the fat layer will remain a buff color and the purple antigen will be distributed throughout the milk. The milk ring test is prone to false reactions caused by abnormal milk derived from

mastitis, colostrum and milk from late in the lactation cycle. Still, in spite of its problems, it may be used as an inexpensive screening test in conjunction with other tests [53]. The milk ring test is prone to false reactions caused by abnormal milk derived from mastitis, colostrum and milk from late in the lactation cycle. Still, in spite of its problems, it may be used as an inexpensive screening test in conjunction with other tests [17, 54]

In spite of the number of reagents required for the complement fixation test and its technical complications, it is a widely used confirmatory test for brucellosis. The basic test consists of *B. aborts* antigen, usually whole cells, incubated with dilutions of heat inactivated (to destroy indigenous complement) serum and a titrated source of complement, usually guinea pig serum. After a suitable time, a pre-titrated number of sheep erythrocytes coated with rabbit antibody is added. If a primary immune complex (*B. aborts* cells and test serum) is formed due to the presence of certain antibody isotypes in the serum, complement was activated and therefore not available to react with the secondary immune complex of sheep erythrocytes and rabbit antibody, resulting in no or only slight lysis of the erythrocytes. Alternately, if no primary immune complex was formed, complement would cause all the sensitized sheep erythrocytes to lyse. The complement fixation test is technically challenging because a large number of reagents must be titrated daily and a large number of controls of all the reagents is required. It is also an expensive test again because of the large number of reagents needed and because it is labor intensive. However, since only IgG1 isotype of antibody fixes complement well, the test specificity is high. Unfortunately, the test does not allow for discrimination of *B. aborts* S19 derived antibody. Other problems include the subjectivity of the interpretation of results, occasional direct activation of complement by serum (anticomplementary activity) and the inability of the test for use with hemolyzed serum samples. In spite of the shortcomings, the complement fixation test has been and is a valuable asset as a confirmatory test in control/eradication programs [17]. Competitive ELISA were developed in order to overcome some of the problems arising from residual *B. aborts*S19 vaccine antibody and from cross reacting antibody. By selecting a monoclonal antibody with slightly higher

Molecular Technique: Molecular biology as a diagnostic tool is advancing and will soon be at the point of replacing actual bacterial isolation. 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. Applications for PCR methods range from the diagnosis of the disease to characterization of field isolates for epidemiological purposes including taxonomic studies. PCR-based assays are also useful in chronically infected patients where the yield of bacteria from blood cultures is usually low. It is rapid, safe and cost effective, the only real problems being some uncertainties regarding specificity [17]. 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. aborts* 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 [54].

Management Strategies

Treatment of Brucellosis in Both Animal and Human: Antibiotic treatment of known infected animals, or of those which are potentially exposed to Brucellae agents, has not been commonly used and it should be ruled out as an option in the control of brucellosis. A limited number of studies have shown rapid reductions in the incidence of brucellosis when the herd of flock was treated but this procedure is considered to be restricted in practice. Treatment has been used in animals of special breeding value, but because of the uncertain outcome it is not generally recommended [52, 55].

The essential element in the treatment of human brucellosis is the administration of effective antibiotics for an adequate length of time. Antibiotic treatment should be implemented at as early a stage as possible, even in patients who appear to be showing a spontaneous improvement. In those patients with complications, additional treatment, including in some cases surgical intervention, will be necessary. A variety of antimicrobial drugs have activity in vitro against Brucella species; however, the results of routine susceptibility tests do not always correlate with clinical efficacy. Consequently, beta-lactam antibiotics, such as penicillins and cephalosporins and macrolide antibiotics, such as erythromycin, are associated with unacceptably high rates of relapse when used to treat patients with brucellosis. Although newer macrolides, such as azithromycin and clarithromycin are more active in vitro than erythromycin, they have not shown superiority over current regimens for treatment of patients with brucellosis and their role in therapy remains to be determined. Doxycycline with gentamicine or repampin used for treating patient more than eight years of old [56].

Prevention and Control: In endemic areas, control of brucellosis is the first challenge. The only way to control human brucellosis is to control the animal disease and stop passage to man. Brucellosis has been controlled or even eradicated in a small number of wealthy countries, by long and costly programs of animal vaccination followed culling of infected animals at later stages. Food hygiene, especially pasteurization of milk is of great importance to prevent human infections. Excellent reviews by Blasco discus this in detail. Control of a disease such as brucellosis requires a 'One Health' approach [57]. Animal and human health must work together with the livestock holders and programs established inform and educate the population at risk. Strong implication of political decision makers is essential. If not yet established, surveillance of human and animal populations should be implemented. Vaccination programs need good vaccines [5].

Two live vaccines, B. melitensis Rev. 1 and B. aborts S19 have been used over past decades with great success for, respectively, small ruminant and bovine brucellosis control programs throughout the world. B. aborts RB51 is also proposed as a vaccine for bovine brucellosis to be used in the final stages of control programs in conjunction with test and slaughter [58].

None of the available vaccines are perfect; they cause abortion in target and non-target animals, can be shed by immunized animals and all can cause brucellosis in human. RB51 is also resistant to rifampicin, one of the drugs of choice to treat human brucellosis. We need new effective vaccines that are safe for both animals and human. There are many projects aiming to improve the efficiency and safety of existing vaccines and to develop new vaccines. There is currently an international call for development of a new brucellosis vaccine with a substantial prize for the first new vaccine licensed [59]

Public Health and Economic Importance of Brucellosis

Public Health Importance of Brucellosis: Brucellosis, particularly B. melitensis is thought to be one of the most prevalent re-emerging zoonotic diseases globally with an estimated incidence of more than 50, 000 human cases per year [59]. The zoonotic importance of brucellosis as zoonosis is increasing owing to tremendous increase in global trade in animal products, rapid deforestation, unplanned and unsustainable development, urbanization, intensive farming, having migratory/nomadic animal husbandry and increased international tours and travel [58, 60]. Even the exhaustive and advanced surveillance and control measures have not been able to reduce the prevalence of brucellosis in most of the developing countries due to poor hygiene, lack of sanitation, poverty, lack of proper administration and political will Halling *et al.* [22]. Brucellosis badly affects livestock welfare and economy. The collective economic losses are the cumulative effect of reduction in the production of milk, abortions, losses of newborn calves resulting from abortions and stillbirths, culling of brucellosis affected animals, hindrance in export and trade of animals, loss of human effort in terms of man-days wasted, veterinary and medical expenses, administrative and governmental expenses on research and control programs [61].

Brucellosis patients as well as their family members should be screened regularly in endemic areas [62, 63]. Incidence of human brucellosis varies from <0.01 to >200 per 100, 000 population in endemic areas globally [64]. Six countries comprising of Syria, Saudi Arabia, Oman, Jordan, Iran and Egypt have accounted for more than ninety human brucellosis case reports annually in 1990 [64].

Brucellosis results in colossal economic losses worldwide both in terms of animal health and production as well as from public health aspects in terms of cost of treatment along with loss of productivity. Bovine brucellosis results in economic losses in countries of Latin America to the tune of approximately US $600 million [65]. The cost of national brucellosis control and eradication program in USA was of the tune of US $3.5 billion during the year 1934 to 1997 and the cost due to reduction in milk yield and abortions in 1952 alone was estimated to be US $400 million [22]. Investigated the link of socio-economic factors and Brucella prevalence in Sri Lanka. Socio-economic parameters like income of family, education level of family members, ethnicity affiliation, experience in farming and advanced [66]. Economic impact of brucellosis several countries fail to recognize the economic importance of brucellosis. As different review shows, the most adverse effect of brucellosis on the livestock population seems to be abortion, followed by stillbirth, infertility and lower milk yields and lastly a longer calving interval [64].

Economic Importance of Brucellosis: Estimation of economic losses Very few countries tackled this chapter on estimating economic losses, no doubt owing to lack of data on funding brucellosis control and on assessing direct economic losses and loss of earnings. In African country like Algeria, Gabon, Mauritania, Morocco, the Democratic Republic of Congo, Tanzania and Tunisia gave a few indications regarding the annual cost of brucellosis control [67]. The countries receive public or private financing (livestock producers).

Table 1: Prevalence of Bovine Brucellosis in different parts of Ethiopia

Study Area	Prevalence	References
Tigray Region	3.19%	69
East Showa Zone, Oromia	11.2%	70
Jijiga Zone	1.38%	71
East Wollega Zone	1.9%	72
Arsi zone	0.05%	73
Southern And Eastern Ethiopia	3.5%	74
Jimma Zone	3.1%	75
Central Oromia	2.9%	76
Addis Ababa	10%	78, 79
Northwestern Part of Ethiopian	14.96%	80
Southwestern	0.77%	81
North Gonder zone	1.4%	82

The public financing amounts to brucellosis is 19459.13 EUR in Swaziland, 20890 EUR in Tanzania and 1897288 EUR in Algeria. In Swaziland, economic losses arising from abortion total 2900023 EUR, while milk losses are assessed at 272210 EUR and export losses at 47384 EUR. Tunisia and the Democratic Republic of Congo reported economic losses from abortion, reduced agricultural manpower and lower milk yields, although they provide no financial evaluation of the losses [20].

Human cases are reported in 11 countries (Algeria, Eritrea, Guinea, Guinea-Bissau, Kenya, Morocco, Mauritania, Niger, Sudan, Tanzania and Tunisia), mainly as a result of consuming raw milk or infected soft cheese, or of contact with infected animals or the placenta or aborted fetus during Brucella-induced abortion. The people at risk are primarily livestock producers, as well as butchers and veterinarians [68]. After noticing the infection, patients are often admitted to hospital and treated using antibiotics, or else they consult traditional medical practitioners, as in Guinea-Bissau, or forego all forms of treatment as it is too expensive. For instance, the cost of treating a patient ranges from 9 EUR in Tanzania to 200 euros in Morocco and as much as 650 euros en Algeria [27].

Status of Brucellosis in Ethiopia: Studies on the prevalence of brucellosis have been carried out in many parts of Ethiopia by different persons. These studies were conducted in local and cross breed animals. In Ethiopia, seroprevalence of bovine brucellosis were reported in areas like Eastern Ethiopia & Guto Gida District East Wollega Zone (1.97 %), East Showa Zone (11.2%), Tigray Region (3.19), Jimma Zone (3.1%), Central Oromia, (2.9%), Arsi Zone (0.05%), Agro-Pastoral Areas and Southern (3.5%) and Jijjiga (1.38%) Addis Ababa (10%), Northwestern Part of Ethiopian (14.96%), Southwestern (.77%), North Gonder zone (1.4%), [69-82].

Moreover, the study reported by only using the screening test for brucellosis affects the result of the study to be reported. Therefore, several factors such as animal management, time difference, type and place of study and laboratory techniques may also contribute for the high or less prevalence of the disease.

CONCLUSION AND RECOMMENDATIONS

Brucellosis is a highly contagious zoonotic disease and a cause of significant reproductive losses in livestock. The disease transmitted to human through consuming contaminated and unpasteurized milk, milk products or by direct contact with infected animals or animal carcasses. In human the main clinical presentations are acute febrile illness, with or without signs of localization and chronic infection. Animal brucellosis causes direct socio-economic effects in communities who depend on animal production for their livelihood. Losses in animals are attributed to direct effects on their offspring due to abortion, stillbirth and infertility whereas indirect losses are due to reduction in milk yields and human suffering resulting from the disease. The disease causes colossal economic losses globally in terms of reduced animal health and production and effect on public health, yet robust surveillance, prevention and control measures are lacking.

Based on the above conclusion, the following recommendations are forwarded as:

- Application of multi-disciplined collaborative approach for effective disease control and prevention as well as to alleviate the economic losses and public health threat caused by brucellosis.
- Due consideration should be taken around researches area for all animal species to limit the transmission dynamics of the disease in between animal species and human.
- All researches related to the disease should be supported by the gold standard diagnostic approaches that empower us to currently the most wide distributed strains of Brucella agents both in human and animal.

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