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Review Article

Review on Molecular Epidemiology and Public Health Significance of Brucellosis

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Abstract

Brucellosis is an important livestock and human disease in many developing countries for its cause of reproductive disease, characterized by abortion, retained fetal membranes and impaired fertility. The genus *Brucella* currently composed of eight terrestrial species and at least two marine species. Terrestrial *Brucella* species include *B. abortus, B. melitensis, B. suis, B. ovis, B. canis,* B. neotomae and two new species, *B. microti* and *B. inopinata. Brucella* isolated from marine mammals is *B. ceti* and *B. pinnipedialis. Brucella* species can invade epithelial cells of the host, allowing infection through mucosal surfaces, in which the outcome of infection dependent on the species of *Brucella* and host. Though its distribution is worldwide; yet brucellosis is more common in countries with poorly standardized animal and public health program and also bio varieties of *Brucella* vary with respect to geographic region. The prevalence of brucellosis depends on different risk factors including host risk factors, agent risk factors, management risk factors and occupational risk factors. Genetically, all *Brucella* species are highly related to each other, exhibiting sequence similarity values of 98% to 100% at nucleotide level (core genome). Despite this close genetic relatedness, the various species can be distinguished from each other by application of high resolution molecular typing tools such as polymerase chain reaction, single nucleotide polymorphism analysis and multi-locus sequence typing or multi-locus sequence in addition to assessment of phenotype and host preference. Each year half a million case of brucellosis occurs in humans around the world. Five out of nine known *Brucella* species can infect humans. The most pathogenic and invasive species for human are *B. melitensis, B. abortus* and *B. canis.* Understanding the *Brucella* species and bio variant through advance knowledge of molecular epidemiology has significant role in the elucidating the source of infection, disease transmission pattern and furthermore in designing specific control strategies through utilization of relevant vaccine in affected livestock population. Prevention and control of brucellosis can be adopted realistically through understanding of local and regional variations in animal husbandry practices, social customs, infrastructures and epidemiological patterns of the disease and species of *Brucella*. Hence, this seminar paper attempted to highlight the molecular epidemiology and public health significance of brucellosis in livestock and human populations.

Keywords: Brucellosis; Diagnosis; Livestock; Molecular epidemiology; Zoonosis

Introduction

Brucellosis is an important livestock and human disease in many developing countries. It is primarily a reproductive disease, characterized by abortion, retained fetal membranes and impaired fertility [1].

Brucellosis is one of the major common bacterial zoonosis in the world caused by organisms belonging to the genus *Brucella*, gram-negative, non-motile and facultative intracellular pathogens that can infect many species of animal of economic importance, such as cattle, sheep, goats, pigs and marine animals. The genus *Brucella* currently composed of eight terrestrial species and at least two marine species. Terrestrial *Brucella* species include *B. abortus, B. melitensis, B. suis, B. ovis, B. canis,* B. neotomae and two new species *B. microti* and *B. inopinata. Brucella* isolated from marine mammals are *B. ceti* and *B. pinnipedialis* [2].

The disease is an old one that has been known by various names, including mediterranean fever, malta fever, gastric remittent fever, and undulant fever (because of the relapsing nature of the fever associated with the disease). Humans are accidental hosts, but brucellosis continues to be a major public health concern worldwide and is the most common zoonotic infection [3].

Human brucellosis is a zoonotic disease with a major impact on public health, even though successful eradication and control programs for domestic animals have been established in many countries around the world. The disease primarily presents as fever of unknown origin with multiple clinical signs and symptoms. Patients regularly suffer serious focal complications such as spondylitis, neurobrucellosis or endocarditis [4]. As the ultimate source of human brucellosis is direct or indirect exposure to infected animals or their products, prevention must be based on elimination of such contact. The obvious way to do this elimination of the disease from animals is often beyond the financial and human resources of many developing countries. For instant, the technical and social difficulties involved in eradicating *B. melitensis* from small ruminants have even taxed the resources of some developed countries. In many situations there is little alternative but to attempt to minimize impact of the disease and to reduce the risk of infection by personal hygiene, adoption of safe working practices, protection of the environment and food hygiene [5].

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Presumptive diagnosis can be made by the use of several specific serological tests to making the diagnosis of *Brucella* antibodies, but unequivocal diagnosis requires the bacteriological demonstration of the organism. Hence, the collection and shipment of appropriate samples to the laboratory have great importance. The diagnosis of brucellosis is usually performed by a combination of methods. The identification of *Brucella* culture relies upon a great deal of phenotypic traits such as requirement for CO_2, phage typing and metabolic tests, which among other problems involves time, bio safety, trained personnel and somewhat ambiguous results. *Brucella* species and biovars have been characterized by conventional phenotypic and serological methods, although such methods are not always reliable [6].

Accurate species delineation can be achieved by conventional multiplex polymerase chain reaction, single nucleotide polymorphism analysis and multilocus sequence typing or multilocus sequence analysis. Highly discriminatory multilocus variable number of tandem repeats analysis allows both species delineation and differentiation of individual isolates and thus represents a perfect first-line tool for molecular epidemiological studies within outbreak investigations [7]. To date, advanced molecular technologies have not been widely used in low income countries where brucellosis is endemic in livestock and humans. Thus, information on the prevailing *Brucella* species, biovars, and genotypes/strains in such areas of endemicity may shed new light on the epidemiology of *Brucella* infection and the species and biovars circulating [8].

Therefore, the objectives of this seminar paper are:

1. To review the molecular epidemiology of brucellosis and
2. To indicate the economic and public health significance of brucellosis.

Etiology

Brucellosis is an important zoonotic disease caused by infection with bacteria of the Genus *Brucella*. It was first isolated by Bruce in 1887 from the spleens of soldiers dying of mediterranean fever on the island of Malta. Bruce called it Micrococcus melitensis. The origin of the disease remained a mystery for nearly 20 years until it was discovered that goats were the source of infection for human populations. Nine *Brucella* species are currently recognized (Table1). Seven of them that affect the terrestrial are *B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae,* and *B. microti,* and two that affect marine animals are, *B. ceti* and *B. pinnipedialis*. The first three species are called classical *Brucella* and within these species, seven biovars are recognized for *B. abortus*, three for *B. melitensis* and five for *B. suis*. The remaining species have not been differentiated into biovars [9].

Organism	Host
B. melitensis	Sheep, Goat and Camel
B. abortus	Buffalo, Cows and Camels
B. canis	Dog
B. suis	Pig
B. neotomae	Rodent
B. ovis	Sheep
B. pinnipediae	Marine animals
B. cetaceae	Marine animals

Table 1: *Brucella* species and their hosts.

Source: [10].

Pathogenesis

The ability of *Brucella* species to cause disease requires a few critical steps during infection. *Brucella* species can invade epithelial cells of the host, allowing infection through mucosal surfaces: Macrophage cells in the intestine have been identified as a portal of entry for *Brucella* species. Once *Brucella* species have invaded, usually through the digestive or respiratory tract, they are capable of surviving intracellularly within phagocytic or non-phagocytic host cells [11].

Brucella has the ability to interfere with intracellular trafficking, preventing fusion of the *Brucella*-containing vacuole with lysosome markers, and directing the vacuole towards a compartment that has rough endoplasmic reticulum which is highly permissive to intracellular replication of *Brucella* [12]. The outcome of infection is dependent on the species of *Brucella* and host. The *Brucella* species that infect livestock are host restricted. For instance *B. melitensis, B. abortus, B. suis* and *B. ovis* infect preferentially small ruminants, cattle, pigs and sheep respectively. With the exception of *B. ovis,* these *Brucella* species have zoonotic potential, with *B. melitensis* being the most pathogenic for humans [13].

Brucella spp lack classical bacterial virulence factors such as exotoxins, cytolysins, a capsule, fimbriae, flagella, plasmids, lysogenic phages, endotoxic lipopolysaccharide, and inducers of host cell apoptosis [14]. However, LPS plays an important role in *Brucella* virulence because it prevents complement-mediated bacterial killing and provides resistance against antimicrobial peptides such as defenses and lactoferrin [15]. Another important virulence mechanism of *Brucella* is the BvrR/BvrS two-component regulatory system, which is required for modulation of the host cell cytoskeleton upon *Brucella* invasion, and for regulation of the expression of outer membrane proteins, some of which are required for full virulence [16]. Cyclic β-1, 2-glucans, which are also part of the outer membrane, is also required for intracellular survival of *Brucella* [17]. Figure 1 shows major events in the pathogenesis of brucellosis and the host immune response.

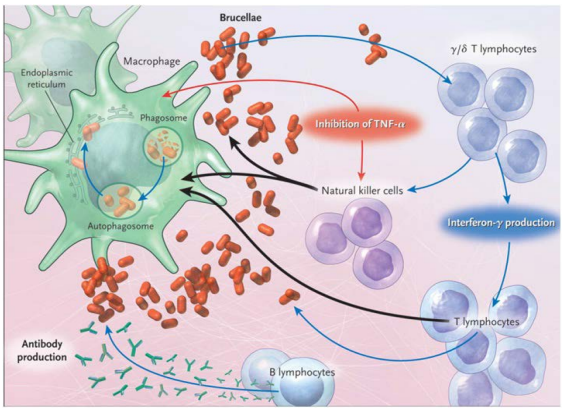

Figure 1: Major events in the pathogenesis of brucellosis and the host immune response.

Source: [18].

Diagnostic Methods of Brucellosis

Bacteriological diagnosis

Although there are much diagnostic method of *Brucella* species,

isolation and then culture the organism is golden standard test up to now. Conventional bacterial culture methods are still used most often to identify *Brucella* and require usually two weeks. Most of this method involves some principal stages for isolation and identification of *Brucella*: Enrichment, selective isolation, and cultivation. Enrichment is used to encourage the growth of very small numbers of *Brucella* or to allow the recovery of injured *Brucella* cells. Likewise, selective enrichment is used to allow additional expansion of the *Brucella* and used to obtain isolated colonies, each derived from a single cell. Finally, colonies with appearances characteristics of *Brucella* are subjected to biochemical tests and other phenotyping techniques to confirm their genus and serotype identity [18].

"The gold standard" for laboratory detection of *Brucella* and species identification is based largely on bacterial isolation and phenotypic characterization. Isolation of *Brucella* organisms from the suspected animal is the golden standard in terms of specificity. However, this method has a limited sensitivity, expensive, time consuming, labor-intensive and has been associated with a heightened risk of laboratory-acquired infection and has the added difficulty of being unpractical to apply on a large scale in control. Polymerase chain reaction is becoming very useful and considerable progress has been made to improve their sensitivity, specificity, and technical case and to lower costs. Nucleic acid amplification has been explored for rapid detection and confirmation of the presence of *Brucella* species [19].

Serological diagnosis

The most common serological tests used in Brucellosis are serum agglutination test, Rose Bengal plate test, and indirect enzyme linked immunosorbent assay [20]. Milk ring test detects milk *Brucella* antibodies and tests only possible on lactating animals. Only applicable on entire herd and yields a rough picture of the status of infection and very uncertain at individual animal level. It has some drawbacks like less reliability in large herds and cannot be used for male animal [10].

The standard Rose Bengal and Complement Fixation tests are the main serological tests used to detect antibodies against *B. abortus* and *B. melitensis*. Both tests have been used for several decades, proving to be successful for eradicating bovine brucellosis in some countries. Nevertheless, there is evidence that both tests are significantly less effective for the diagnosis of brucellosis in sheep and goats than in cattle [20].

Complement fixation test is a widely used confirmatory test for brucellosis. It 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. Some of the problems of CFT are few positive reactions, sometimes negative result in early stage of infections, the test is rather expensive and complicated. Other problems include the subjectivity of the interpretation of result occasional direct activation of complement by serum (anti complementary activity) and the inability of the test for use with hemolyzed serum samples. False positive results may also occur in animals infected with organisms antigenically related to *Brucella* [10].

ELISA is very sensitive and good for detecting latent carriers, incomplete antibodies, relatively simple and easily automated. A very good as control test in free areas and as survey testing areas where no vaccination have been performed, but complicated and cannot be carried out everywhere, severely hampered by vaccination and still too little standardized. Indirect Enzyme-Linked Immunosorbent Assay have been developed using purified smooth lipopolysaccharide as the antigen and have been reported to be at least as sensitive and specific as the combination of both RBT and CFT for the diagnosis of brucellosis in ruminants [21].

Molecular diagnosis: PCR, RFLP and MLVA

Genetically, all *Brucella* species are highly related to each other, exhibiting sequence similarity values of 98% to 100% in aligned regions (core genome). The population structure is clonal. Despite this close genetic relatedness, the various species can be distinguished from each other by application of high resolution molecular typing tools, in addition to assessment of phenotype and host preference. Accurate species delineation can be achieved by conventional multiplex polymerase chain reaction single nucleotide polymorphism analysis and multilocus sequence typing or multilocus sequence analysis.

Highly discriminatory multilocus variable number of tandem repeats analysis allows both species delineation and differentiation of individual isolates and thus represents a perfect first-line tool for molecular epidemiological studies within outbreak investigations [22]. Figure 2 shows difference of *B. melitensis* 16M with five *Brucella* species genomes by microarray.

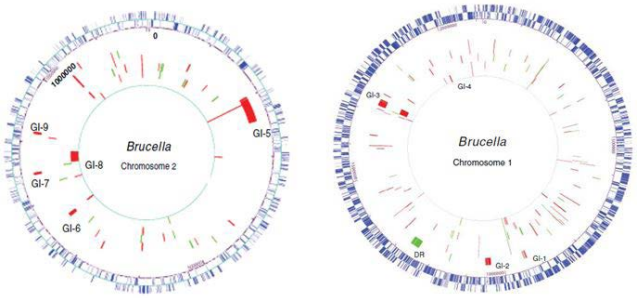

Figure 2: Comparison of five *Brucella* species genomes to *B. melitensis* 16M by microarray.

Source: [23].

Polymerase chain reaction is an *in vitro* technique for the nucleic acid amplification, which is commonly used to diagnose infectious diseases. The use of PCR for pathogens detection, genotyping and quantification has some advantages, such as high sensitivity, high specificity, reproducibility and technical ease. Brucellosis is a common zoonosis caused by *Brucella* spp., which still remains as a major health problem in many developing countries around the world. The direct culture and immunohistochemistry can be used for detecting infection with *Brucella* spp. However, PCR has the potential to address limitations of these methods. PCR are now one of the most useful assays for the diagnosis in human brucellosis [24].

One of the first PCR assays to differentiate among *Brucella* species was called *abortus-melitensis-ovis-suis* PCR, developed by Bricker and Halling in 1994. This PCR uses a single reverse primer, targeting the *Brucella* specific insertion element IS711, and four different forward primers, each specific for a given species as estimated by testing representative isolates. Species are differentiated on the basis of

different PCR fragment sizes. In 2006, a new conventional multiplex PCR (Bruce-ladder), using eight primer pairs in a single reaction, was developed by García-Yoldi and colleagues [25]. Because this PCR covers all species and biovars it rapidly replaced the AMOSPCR as a diagnostic tool and is still used in many diagnostic laboratories. The most recent multiplex PCR assay to differentiate among *B. suis* biovars 1 to 5 (Suis-ladder) was developed in 2011 by scientists [26].

The first Multiple-Locus Variable number tandem repeat Analysis (MLVA assay) named 'HOOF-Prints' (hypervariable octameric oligonucleotide fingerprints), was developed by Bricker et al., in [27]. The *Brucella* genome contains a family of tandem repeats sharing the repeat unit 'AGGGCAGT'. Eight highly variable such loci, present in most *Brucella* species, were selected for use in the HOOF-Print assay. Variations of the repeat numbers at each locus can easily be investigated by amplifying the corresponding regions and subsequent gel electrophoresis or, preferably, capillary electrophoresis, given the short repeat unit size. This selection of tandem repeats has a very high discriminatory power and can be useful for local outbreak investigations. However, it cannot provide a species assignment owing to the high level of homoplasy at these loci. With the HOOF-Print assay a reliable tool to study the relationship of human cases and outbreak dynamics became available for the first time. Indeed, high resolution markers allow the discrimination of individual strains and therefore can be used for trace-back analyses and epidemiological studies in outbreak scenarios. A high discriminatory power is desired when investigating an outbreak with very limited geographical and temporal distribution, and highly variable loci will then be preferred. However, rapidly evolving Variable-Number Tandem-Repeat (VNTR) markers often suffer from homoplasy, i.e., the appearance of the same genetic alteration in two or more branches of a phylogenetic tree. These phenomena can disrupt and confound the accurate phylogenetic placement of some isolates within an MLVA cluster and prevent accurate species-level designation [28].

None of the existing molecular tools provide adequate resolution to confidently permit epidemiological trace back in the case of accidental import or deliberate release. However, the completion of genome sequences for a *B. suis* and a *B. melitensis* strain provided an opportunity to assess the presence of tandem repeats that might facilitate the development of an MLVA scheme. Initial analysis indicated the presence of many potentially useful regions of diversity in the *Brucella* genomes and, indeed, during the planning stages of the present study, an MLVA scheme that utilizes eight distinct copies of an octameric repeat (the "HOOF-Prints" assay) was described [26].

Several molecular typing methods are introduced to find DNA polymorphism that is able to identify the *Brucella* species and biovars, among which detection of polymorphisms by PCR-RFLP has several advantages including the easy implementation, interpretation and use for large quantities of samples. In this method, by Omp25, Omp2 and Omp31 loci and all *Brucella* species can be differentiated and their biovars identified. Several studies use these genes to differentiate *Brucella* species and biovars performed around the World [29]. The genus *Brucella* has ten recognized species with more than 90% DNA homology. These species cause brucellosis that is of economic and public health importance in terrestrial and aquatic animals and humans [30].

Risk Factors for Brucellosis

Host risk factors

Brucellosis infects a variety of domestic and wild animals and man causing incapacitating disease. The susceptibility of animal to *Brucella* infection is influenced by the age, breed and pregnancy status [31]. Sexually mature animals are much more susceptible to infection, regardless of gender. Younger animals tend to be more resistant to infection. Herd size and animal density are directly related to prevalence of the disease and difficulty in controlling infection in the population. 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 [10].

The predilection sites being the reproduction tract of male and female especially the pregnant uterus. Allatoic factors stimulate the growth of most *Brucella*. These factors include Erythritol, possibly steroid hormones and other substances. Erythritol is present in the placenta and male genital tract of cattle, sheep, goats, and pigs but not in humans [32]. Female usually abort only once, after which a degree of immunity develops and the animals remain infected and large number of *Brucella* be expelled in the fetal fluids at subsequent parturition [31]. Cattle susceptibility to *B. abortus* infection is influenced by age, sex, breed and reproductive status of the individual animal [33].

Agent risk factors

B. abortus 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. The bacterium possesses an unconventional non-endotoxin lipopolysaccharide, 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 [10].

Naturally infected animals and those vaccinated as adults with strain 19 remain positive to the serum and other agglutination tests for long periods. The antibody response to *Brucella* consists of an early IgA and IgM is a type response, the timing of which depends on the route of exposure, the dose of bacteria and the health status of the animal. The IgM response is followed shortly by production of IgG1 antibody and later by IgG2 [34].

The total concentration of IgG2 increases with age. Most cross reacting antibody, resulting from exposure to microorganism other than *Brucella* spp., consist of IgM, making serological tests which measure IgM not specific as false positive results occur, leading to low assay specificity. In the case of *Brucella* infection, the concentration of anti-*Brucella* total IgG2 increases with the level of antigen exposure, therefore the monitoring of IgG1 and IgG2 *Brucella* antibody levels is relevant for detection of *Brucella*-infected cattle [35].

Occupational risk factors

Laboratory workers handling *Brucella* cultures are at high risk of acquiring brucellosis trough accidents, aerosolization and/or inadequate laboratory procedures. In addition to this, abattoir workers, farmers and veterinarians are at high risk of acquiring the infection

[36]. Acquiring infection through direct contact is a potential threat to occupational groups such as farmers, veterinarians, butchers, laboratory workers, milkers and inseminators. Handling aborted materials or attending retained placenta or dystocia without protective gear is a common practice to most field veterinary assistants, abattoir workers and in many rural pastoral settings. This may suggest that animal health workers and rural communities are also at great risk of contracting the disease if the disease is present in domestic animals [37]. Figure 3 shows way of transmission of *Brucella* to human being.

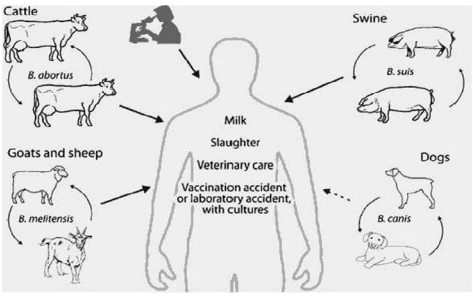

Figure 3: Transmission of *Brucella* to humans.

Source: [37].

Management risk factors

The unregulated movement of cattle from infected herds or areas to brucellosis-free herds or areas is the major cause of breakdowns in brucellosis eradication programs. Once the herds are infected, the time required to become free of brucellosis is increased by large herd size, by active abortion, and by loose housing. 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 [10].

Molecular Epidemiology of Brucellosis

Though its distribution is worldwide; yet brucellosis is more common in countries with poorly standardized animal and public health program [38]. New *Brucella* strains or species may emerge and existing *Brucella* species adapt to changing social, cultural, travel and agricultural environment. The incidence of reactors in newly established cattle farms may be more than 30% however, the highest rate (72.9%) of infection till now has been reported in the Palestinian Authority [39].

It is interesting to note that the second highest prevalence (71.42%) of brucellosis has been reported in mules from Egypt. Invariably, all domestic animals suffer from this disease. Brucellosis in buffaloes has been reported from Egypt (10.0%) and Pakistan (5.05%). Since cattle are found throughout the world, prevalence of brucellosis (0.85 to 23.3%) in cattle has been reported from a wide range of countries. In camels, brucellosis has been reported from Arabian and African countries (0.0-17.20%), where the disease also occurs in buffaloes, equines and swine. Variable prevalence of this disease has been reported in sheep and goats. Bio varieties of *Brucella* vary with respect to geographic region. *B. melitensis* biovar 1 from Libya, Oman and Israel and *B. melitensis* biovar 2 from Turkey and Saudi Arabia have been isolated. *B. melitensis* biovar 3 is the most commonly isolated species from animals in Egypt, Jordan, Israel, Tunisia and Turkey. *B. abortus* biovar 1 in Egypt, biovar 2 in Iran, biovar 3 in Iran and Turkey and biovar 6 in Sudan have been reported [40].

The countries with the highest incidence of human brucellosis include Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman. Bahrain is reported to have no incidence [41]. The percent prevalence of bovine brucellosis has been reported to decrease in Ireland and Italy during the year 1999-2000 but there had been a trend towards a significant increase in Azores [42].

Characterization of the molecular epidemiology of *B. abortus* is an important component of efforts by APHIS and state animal health agencies to control the disease among wildlife and livestock. One of the initial protocols used for this purpose was the HOOF-Prints assay which exploited the presence of 8-base pare tandem repeat sequences at 8 loci in the *B. abortus* genome. This assay was used to differentiate clusters and groupings among a panel of 97 *B. abortus* reference strains and field isolates, representing three biovars, collected from different geographic locales in the United States [43].

Based on their agglutinating properties with specific antisera, *B. melitensis* can be differentiated into three biovars, biotypes 1, 2 and 3 of which biotype 1 is known to be present in Peru [8]. Recently, a highly discriminatory method for the genotyping of *Brucella* known as MLVA analysis has become available. This method makes use of various loci on the *Brucella* genome that are composed of repeats of short nucleotide sequences. These tandem-repeat units tend to occur in various numbers, and various alleles can be observed in different species and isolates. The recently published MLVA-16 assay, developed for the genotyping of *Brucella*, makes use of eight mini-satellite loci for species identification, supplemented with a selection of eight more polymorphic microsatellite loci for the further characterization and differentiation of isolates. Whereas the MLVA-16 assay can be used for the biovar classification of *B. abortus* and *B. suis*, no correlation between biovars and genotype has been observed for *B. melitensis* [44].

The MLVA-16 typing of animal and human *Brucella* isolates has revealed that clusters of individual genotypes within a species may show a distinct geographic distribution. For instance, human isolates of *B. melitensis* from Europe and North Africa can be divided according to their geographic origin into a west and an east Mediterranean cluster. Within the west Mediterranean cluster (which includes isolates from France, Switzerland, Tunisia, and Algeria), a clearly separate cluster originating from Italy can be identified. Genotypes are relatively stable, and isolates with identical MLVA patterns have been obtained from the same geographic area during a time span of almost three decades. A considerable number of distinct *B. melitensis* genotypes already have been identified [20]. MLVA typing additionally has some practical clinical applications, such as tracing sources of infections and discriminating relapse from re-infection [45].

High resolution phenotypic and molecular approaches have been developed for *Brucella* speciation, bio typing, and epidemiological traceback. To date, advanced molecular technologies have not been widely used in low income countries where brucellosis is endemic in livestock and humans. Thus, information on the prevailing *Brucella* species, biovars, and genotypes/strains in such areas of endemicity may shed new light on the epidemiology of *Brucella* infection and the species and biovars circulating. Besides this generic scientific rationale for undertaking such investigations, increased understanding of the *Brucella* epidemiology is critical for refining control of brucellosis in resource weak countries

where the same measures as in high income countries cannot be applied [8]. Table 2 shows the origin of *Brucella* strains and their profiles.

Strain	Biovar	Host	Source
B. abortus UK8/01	1	Human	Eire
B. abortus I12	1	Bovine	Northern Ireland
B. abortus F6/0404376	1	Human	New Zealand
B. abortus R51/03	1	Bovine	United Kingdom
B. abortus 5/93	3	Bovine	United Kingdom
B. melitensis F3/02	2	Human	Norway
B. melitensis 1BM1	1	Not known	Portugal
B. melitensis 63/19	2	Human	India
B. melitensis 66/59	3	Ovine	India
B. melitensis 65/155	3	Ovine	Mongolia
B. melitensis UK19/4	1	Human	Ethiopia
B. melitensis R3-60	1	Livestock	Tanzania
B. ovis 79/60	3	Ovine	France
B. ovis 63/96	3	Ovine	Argentina
B. ovis 81/2	3	Ovine	Germany
B. suis 1330	1	Porcine	Croatia
B. suis F7/03 BSI	2	Porcine	Germany
B. suis 01-5744	2	Porcine	South Africa
B. canis 79/85	1	Canine	South Africa
B. canis 79/92	3	Canine	France
B. canis 79/139	2	Canine	United Kingdom

Table 2: Origins of *Brucella* strains showing allelic profiles and ST designations. Source: [46].

Economic and Public Health Significance

Economic significance

The epidemiology of brucellosis is complex. The important factors that could contribute to the occurrence and spread in livestock include, farming system and practice, farm sanitation, livestock movement, sharing of grazing lands and moderate changes towards identification. Brucellosis occurring worldwide in domestic and game animals as well as humans creates a serious economic problem for the intensive and extensive livestock production systems. Losses in animal production due to this disease can be of major importance primarily because of 20% decreased milk production in aborting cows. The common sequel of infertility increases the period between lactations. A high incidence of permanent infertility results in heavy culling of valuable cows and some deaths occur as a result of acute metritis following retention of the placenta [10].

The economic losses due to bovine brucellosis include: Losses of calves due to abortion, reduced milk yield, culling and condemnation of valuable cows because of breeding failure, endangering animal export trading of a nation, loss of man power, medical costs and government cost for research and eradication programs. Available information indicates that brucellosis is one of the most serious diseases of cattle in Latin America and other developing areas. Official estimates put annual losses from bovine brucellosis in Latin America at approximately US$ 600 million [5].

Brucellosis in sheep caused by *B. ovis* has been reported in Australia, New Zealand the United States, South Africa and Europe. The incidence has been very high in some areas, and there was much economic loss at one time. In California, 30-40% of rams were thought to be affected and annual loss of US $ 2 million was estimated. *B. suis* is a chronic disease of swine manifested by sterility and abortion in sows, heavy piglet mortality and orchitis in boars. The disease owes its economic importance to the fertility and reduction in numbers of pigs weaned per litter that occur in infected herds [10].

Public health significance

Five out of nine known *Brucella* species can infect humans. The most pathogenic and invasive species for human are *B. melitensis, B. abortus* and *B. canis.* The zoonotic nature of marine *Brucella (B. ceti)* has been documented. Human brucellosis caused by *B. melitensis* is the most sever one followed by *B. suis, B. abortus* and *B. canis* in decreasing order. They are listed as potential bio-weapons by the contents for disease control and prevention program in USA. This is due to the highly infectious nature of three species, as they can be aerosolized. Moreover an outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza [47].

Each year half a million case of brucellosis occurs in humans around the world. The prevalence of infection in animal reservoir provides a key of its occurrence in humans [48]. Humans are infected by eating or drinking something that is contaminated with *Brucella*, breathing organisms (in halation or wind infection). The relative importance of etiological agent, mode of transmission and path way of penetration varies with the epidemiological area, animal reservoirs and occupational groups at risk. Conception of sheep and goat milk contain *B. melitensis* is an important source of human-brucellosis worldwide and has caused several out breaks. For exam ple, in some countries including Italy 99% of human brucellosis is caused by *B. melitensis*. In countries where milk and dairy products are always pasteurized, brucellosis principally affects persons who are close contact with animals and animal products [20]. The following map shows incidence of human brucellosis in worldwide (Figure 4).

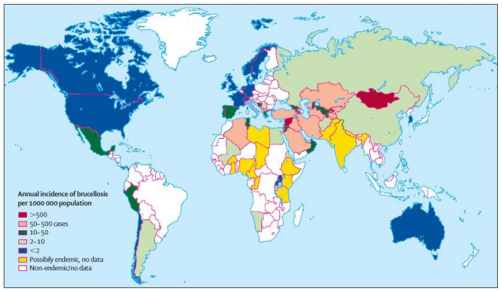

Figure 4: Worldwide incidence of human brucellosis. Source: [2].

Status of Brucellosis in Ethiopia

In Ethiopia, brucellosis was reported in different animal species by various workers. Occurrence of brucellosis in different animal species, traditional management system and custom of consumption of raw or uncooked animal products indicate the need of study of brucellosis in this country. Type of management system was a potential risk factor for sero-prevalence of brucellosis; all sero-positive were from the group of animals kept in the extensive management system [49].

Animal brucellosis in Ethiopia

Cattle

The evidences of Brucella infections in Ethiopian cattle have been serologically demonstrated by different authors. A relatively high seroprevalence of brucellosis (above 10%) has been reported from small holder dairy farms in central Ethiopia while most of the studies suggested a low seroprevalence (below 5%) in cattle under crop-livestock mixed farming. There is a scarcity of published literature on the status of cattle brucellosis in pastoral areas of the country where large population of cattle are reared. So far, a study carried out in east Showa zone of Ethiopia showed a relatively higher seroprevalence in pastoral than agropastoral system [50].

Most of the previous studies on cattle brucellosis have been carried out in central and northern Ethiopia, and do not provide an adequate epidemiological picture of the disease in different agro-ecological zones and livestock production systems of the country. In particular, there is no information on cattle brucellosis across various livestock production systems of southern and eastern part of the country, which gave impetus to the initiation of this study. The present study was therefore aimed at determining the prevalence of cattle brucellosis and associated risk factors across the two livestock production systems, pastoral and crop-livestock mixed systems, in Southern and Eastern Ethiopia [49]. There is summary on prevalence of brucellosis in Ethiopia by using RBPT and CFT in table 3.

Locations	Breed	Number of Animals	Prevalence (%)
Tigray	Cross	816	3.19
Bahir Dar	Cross	1135	0.26
Sidama Zone	Cross	811	2.5
Local		1627	1.7
Jimma Zone	Cross	805	0.8
Local		1305	0.2
Northwest Ethiopia	Cross	4243	22

Table 3: Summary on prevalence of brucellosis in Ethiopia by using RBPT and CFT. Source: [9].

Human brucellosis in Ethiopia

As compared to study of animal brucellosis, study of human brucellosis in Ethiopia is sparse with even less information on risk factors for human infection. For instance, out of 56 cases with fever of unknown origin, two (3.6%) were reported to be positive for *B. abortus* antibodies by RBPT and CFT [51]. A study conducted in traditional pastoral communities by Ragassa et al., [27] using *B. abortus* antigen revealed that 34.1% patients with febrile illness from Borena, 29.4% patients from Hammer and 3% patients from Metema areas were tested positive using *Brucella* IgM/IgG Lateral Flow Assay. But they failed to include a parallel study of animal brucellosis. Studies conducted in high risk group such as farmers, veterinary professionals, meat inspectors and artificial insemination technicians in Amhara Regional State [52] and Sidama Zone of Southern People Nations and Nationalities Sate [53].

Control and Prevention

In animals

Prevention and control of brucellosis can be adopted realistically through understanding of local and regional variations in animal husbandry practices, social customs, infrastructures and epidemiological patterns of the disease. The common approaches used to control brucellosis include, quarantine of imported stock, hygienic disposal of aborted fetuses, fetal membrane and discharges with subsequent disinfection of contaminated area. Animals which are in advanced pregnancy should be kept in isolation until parturition. Moreover replacement stock should be purchased from herd free of brucellosis, and decide for or against immunization of negative animals. Eradication by test and slaughter of positive reactors is also possible [54].

Test and isolation/slaughter

The decision about slaughter of test-positive animals is made after regulatory, economic and prevalence factors are considered. In most cases, test and slaughter of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (e.g., 2%). Retention of positive animals is less hazardous if the remaining animals have been vaccinated but should only be considered as a last resort. The isolation of test-positive animals is essential, especially during and after parturition. The immediate slaughter of test-positive animals is expensive and requires animal owner cooperation. Compensation is usually necessary. Furthermore, the application of test and slaughter policies is unlikely to be successful with brucellosis of sheep and goats where the diagnostic tests are less reliable than in cattle [55].

Hygiene

The goal in the application of hygiene methods to the control of brucellosis is reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control. Factors such as the methods of animal husbandry (e.g., commingling of herds or flocks), patterns of commerce, prevalence of clinical signs, type of facilities, and degree of dedication of the owners of animals, will also determine success. Owners are often poorly informed about disease transmission and recommendations, such as separation of parturient animals, can be difficult or impossible to implement [56].

Control of animal movement

Animals should be individually identified by brand, tattoo or ear tag. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, importations into clean areas must be restricted to animals that originate from brucellosis-free areas, that have a herd/flock history of freedom from the disease and that have given negative reactions to recently performed diagnostic tests [54].

Vaccination

There is general agreement that the most successful method for prevention and control of brucellosis in animals is through vaccination. While the ideal vaccine does not exist, the attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats and *B. abortus* strain 19 have proven to be superior to all others. The non-agglutinogen

B. abortus strain RB51 has been used in the USA, Canada and some Latin American countries, South Africa and Egypt with encouraging results. The source and quality of the vaccines are critical. The dosages and methods of administration, especially with Rev.1, vary and these can affect the results [20].

It is often recommended that vaccination with strains 19 and Rev.1 should be limited to sexually immature female animals. This is to minimize stimulation of post vaccinal antibodies which may confuse the interpretation of diagnostic tests and also to prevent possible abortions induced by the vaccines. However, field and laboratory studies have demonstrated that conjunctival administration of these vaccines makes the vaccination of the herd or flock a practical and effective procedure. Rapid herd immunity is developed and application costs are minimized. The lowered dose results in lower antibody titers and serenade rapidly. Several diagnostic tests have been developed which are useful in differentiating antibody classes. Of these, the complement fixation test and ELISA are currently the most widely used [55].

In human

The most rational approach for preventing human brucellosis is control and eradication of the infection in animal reservoirs. In addition there is a need to educate the farmers to take care in handling and disposing of aborted fetus, fetal membrane and discharges as well as not to drink unpasteurized milk and abattoir workers in transmission of infection especially via skin abrasion [57].

Treatment

Due to intracellular localization of *Brucella* and its ability to adapt to the environmental conditions encountered in its replicative niche e.g., macrophage [58] treatment failure and relapse rates are high and depend on the drug combination and patient compliance. The optimal treatment for brucellosis is a combination regimen using two antibiotics since monotherapies with single antibiotics have been associated with high relapse rates [3]. The combination of Doxycycline with Streptomycin (DS) is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis [55].

Neither streptomycin nor doxycycline alone can prevent multiplication of intracellular *Brucella*. Although the DS regimen is considered as the gold standard treatment, it is less practical because the streptomycin must be administered parenterally for 3 weeks. A combination of doxycycline treatment (6 weeks duration) with parenterally administered gentamicin (5 mg/kg) for 7 days is considered an acceptable alternate regimen. Although DS combinations had been considered by the WHO to be the standard therapy against brucellosis for years, in 1986 the Joint FAO/WHO Expert Committee on Brucellosis changed their recommendations for treatment of adult acute brucellosis to rifampicin (600–900 mg/day orally) plus doxycycline (200 mg/day orally) DR for 6 weeks as the regimen of choice. However, the studies that compared the effectiveness of DR regimen with the traditional DS combination concluded that DR regimen is less effective than the DS regimen especially in patients with acute brucellosis [56].

Conclusion and Recommendations

Brucellosis is a zoonotic disease caused by a number of *Brucella* species and is characterized by chronic macrophage infection. However, genes that may contribute to intracellular survival of the *Brucella* species are not well studied. The genus *Brucella* includes several species and biovars. The difference between their species and biovars is mainly based on phenotypic characteristics of lipopolysaccharide antigens, sensitivity to colors, need to CO_2, H_2S production, metabolic features and phage typing. But these methods of differentiation are unreliable and their sensitivity is low. Genetically, all *Brucella* species are highly related to each other, exhibiting sequence similarity values of 98% to 100% in aligned regions (core genome); the population structure is clonal. Despite this close genetic relatedness, the various species can be distinguished from each other by application of high resolution molecular typing tools, in addition to assessment of phenotype and host preference. Accurate species delineation can be achieved by conventional multiplex Polymerase Chain Reaction (PCR), Single Nucleotide Polymorphism (SNP) analysis and Multilocus Sequence Typing (MLST) or Multilocus Sequence Analysis (MLSA). Highly discriminatory multilocus Variable Number of Tandem Repeats (VNTR) analysis (MLVA) allows both species delineation and differentiation of individual isolates and thus represents a perfect first-line tool for molecular epidemiological studies within outbreak investigations. The most important advantages of understanding the molecular epidemiology of brucellosis is for identification of the specific corresponding vaccinal strains to be used for the control of the disease in the specific region. Economic losses in animal production due to this disease can be of major importance primarily because of decreased milk production, abortion, sterility and mortality. Each year half a million case of brucellosis occurs in humans around the world. The prevalence of infection in animal reservoir provides a key of its occurrence in the human population specially farmers, veterinarians, butchers, laboratory workers, milkers and inseminators.

Based on the above conclusion the following recommendations are forwarded:

- Isolation and molecular characterization of species and biovars causing brucellosis in livestock and human should be identified for further control of brucellosis using the existing vaccines,
- High sensitive and specific diagnostic tests such as isolation combined with molecular based diagnostic techniques should be utilized for confirmatory diagnosis of brucellosis,
- In case of our country, even though molecular techniques of diagnosis are famous for their sensitivity and specificity, there is no any well-organized molecular diagnostic laboratory. Therefore the government should encourage the development of laboratories that conduct diagnosis at molecular level,
- Individuals at high risk of getting the infection should be well informed about the disease transmission route and proper safety materials and disinfection should be provided.

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