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Original Article

Risk Factors, Molecular Epidemiology and Public Health Importance of Brucellosis

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SUMMARY

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 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. 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.

KEWWORDS: Brucellosis, Molecular epidemiology,public health importance, Risk factors

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1. INTRODUCTION

Brucellosis is a highly contagious zoonotic chronic bacterial disease of public health, wildlife and livestock importance [1]. The disease is caused by twelve species of the Genus Brucella and which are distributed worldwide [2,3].Susceptibility to brucellosis varies among individual animals. It depends on the animals' natural resistance, age, sex, level of immunity and environmental stress [4]. Adult animals are highly susceptible to and both sexes become infected with brucellosis [5] and that large herd size and age of cattle had a significant association with brucellosis seropositivity [6]. 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 [7].

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. Humans are accidental hosts, but brucellosis continues to be a major public health concern worldwide and is the most common zoonotic infection[4]. 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 [8].

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 [9] would be very important.

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 CO2, 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 [10]. 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 [11].

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 [12].Therefore, this review paper was designed with the objectives of: to review the molecular epidemiology of brucellosis andpublic health significance of brucellosis.

2. BRUCELLOSIS

2.1. 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*[13].The origin of the disease remained a mystery for nearly 20 years until it was discovered.Twelve Brucella species are currently recognized. The six classical species are *B. abortus* in cattle, *B. melitensis* in goats, *B. suis* in pigs, *B. canis* in dogs, *B. ovis* in sheep, *B. neotomae* in rat [14, 15] andwithin these species, different numbers of biovars are recognized for *B. abortus*, for instance three for *B. melitensis* and five for *B. suis*. The remaining species have not been differentiated into biovars[13]. Organism's Hostsof *B. melitensis*are Sheep, Goat and Camel;*B. abortus* Buffalo, Cows and Camels;*B. canis* Dog;*B. suis* Pig;*B. neotomaei* Rodent;*B. ovis* Sheep;*B. pinnipediae* Marine animals and*B. cetaceae* Marine animals [2].

2.2. PATHOGENSIS

The ability of Brucella species to cause disease requires a few critical steps during infection. Brucellaspecies 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 intracellular within phagocytic or non-phagocytic host cells [16]. 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 [17]. 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 [18].

Brucella spp. lack classical bacterial virulence factors such as exotoxins, cytolysins, a capsule, fimbriae, flagella, plasmids, lysogenic phages, endotoxin lipopolysaccharide, and inducers of host cell apoptosis[19]. 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 [20].Another important virulence mechanism of Brucella is the 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 [1]. Cyclic β-1, 2-glucans, which are also part of the outer membrane, is also required for intracellular survival of Brucella [21].

2.3. DIAGNOSTIC METHODS OF BRUCELLOSIS

2.3.1.BACTERIOLOGICAL DIAGNOSIS

Although there are much diagnostic method of Brucella species, isolation and then culture of 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 [22].

The gold standard" for laboratory test that detects 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 [23].

2.3.2.SEROLOGICAL DIAGNOSIS

The most common serological tests used in Brucellosis are serum agglutination test, Rose Bengal plate test, and enzymelinked immunosorbent assay (ELISA)[24]. Milk ring test detects milk Brucellaantibodies 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 [2]. 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 [25].

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 [2].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 [26].

2.3.3.MOLECULAR DIAGNOSIS:

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 [27].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. 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 [28].

In 2006, a new conventional multiplex PCR (Bruce-ladder), using eight primer pairs in a single reaction, was developed by García-Yoldi and colleagues. 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 [29]. The first Multiple-Locus Variable number tandem repeat Analysis (MLVA assay) named "HOOFPrints" (hyper variable octameric oligonucleotide fingerprints), was developed by Bricker *et al.*, [30]. 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. 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 [27].

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. 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. Several studies use these genes to differentiate Brucella species and biovars performed around the World [31]. The genus Brucella has twelverecognized 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 [32].

2.4. TREATMENTS

Due to intracellular localization of Brucella and its ability to adapt to the environmental conditions encountered in its replicative niche e.g., macrophage, 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 [33]. 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 [34].

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 (doxycycline with rifampicin) 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 [1].

2.5. CONTROL AND PREVENTION

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 [35].

Test and isolation/slaughter decision of 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 [34].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. 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 the success. Owners are often poorly informed about disease transmission and recommendations, such as separation of parturient animals, can be difficult or impossible to implement [1].

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 [35].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 [24].

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. 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 [36].

3. RISK FACTORS FOR BRUCELLOSIS

2. 1.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 [37]. 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 [2].

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 [38]. 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 [37]. Cattle susceptibility to *B. abortus* infection is influenced by age, sex, breed and reproductive status of the individual animal [39].

2.2. AGENT RISK FACTORS

Brucella 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 nonendotoxin 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 [2].

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 [40].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 Brucellainfection, 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 [41].

2.3. 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 [42]. 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 [43].

2.4.MANAGEMENT RISK FACTORS

The unregulated movements of cattle from infected herds or areas to brucellosis-free herds or areas are 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[2].

4. MOLECULAR EPIDEMIOLOGY OF BRUCELLOSIS

Though its distribution is worldwide; yet brucellosis is more common in countries with poorly standardized animal and public health program [44]. 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 [45].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. 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 [46]. 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 [47].

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 [48]. 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 [49].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 [50].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*[51].

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 [24].

MLVA typing additionally has some practical clinical applications, such as tracing sources of infections and discriminating relapse from re-infection [52]. High resolution phenotypic and molecular approaches have been developed for Brucella speciation, bio typing, and epidemiological trace-back. 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 Brucellaepidemiology is critical for refining control of brucellosis in resource weak countries where the same measures as in high income countries cannot be applied [50].

5. PUBLIC HEALTH SIGNIFICANCE OF BRUCELLOSIS

Six out of twelve 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 [53].

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 [33]. 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 that are at risk. Consumption of sheep and goat milk contain *B. melitensis* is an important source of humanbrucellosis worldwide and has caused several out breaks. For example, 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 [24].

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. 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 [2].

6. CONCLUSION AND RECOMMENDATIONS

Brucellosis is one of the most important priority diseases of livestock and public health importance. It is a zoonotic disease caused by a number of Brucella species and is characterized by chronic macrophageand reproductive infection. There are several factors that responsible for the transmission and maintenance of brucella species being concluded under host related risk factors, agent risk factors, occupational risk factors and management risk factors. However, genes that may contribute to intracellular survival of the Brucella species are not well studied 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.).Brucella species are listed as one of the potential bio-weapons causing severe infection in which its outbreak would be difficult to detect because the initial symptoms are easily confused with those of influenza. Humans can be infected through ingestion of contaminated material with the bacteria, inhalation of the agent and through direct contact with skin abrasion. Hence, control and eradication of the infection in animal reservoirs and awareness creation campaign should be practiced for farmers and professionals who directly make contact with animals and their products. Therefore 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 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.
- Therefore, the government should encourage the development of well-organized molecular diagnostic laboratory
- 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.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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