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BRUCELLOSIS: CURRENT STATUS OF THE DISEASE AND FUTURE PERSPECTIVES

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Abstract: Brucellosis is a transmissible bacterial zoonotic disease caused by Gram-negative coccobacillus bacteria of the genus *Brucella.* The disease severely hinders the livestock industry and human health. In several instances, infected animals act as carriers for the cross-species transmission of brucellosis. Social issues, poor husbandry practices, irregularities in the marketing and movement of domestic animals, and lack of coordination between veterinary and human health services are some of the key factors responsible for the transmission and prevalence of Brucellosis. Human contact with infected domestic animals is often the transmission route of Brucellosis infection. Therefore, human brucellosis could be eradicated globally by eradicating animal brucellosis. This review describes the current status of brucellosis and the risk factors of the disease in animals and the human population. In addition, there is a further discussion of the various issues related to the control and prevention of brucellosis in domestic animals and humans.

Key words: Brucella; brucellosis; epidemiology; serological test, molecular diagnostic test

1. Introduction

Brucellosis is an infectious zoonotic disease of domestic animals, as well as humans, globally [1–3]. Various domestic animals such as camels, goats, sheep, cows as well as humans are affected by brucellosis. It is caused by the *Brucella* species such as *Brucella melitensis* in small ruminants, *Brucella abortus* in cattle, and *Brucella canis* in dogs [4–5] *Brucella* species are Gram-negative, small coccobacillus, slow-growing and intracellular bacteria that are capable of surviving and multiplying within macrophages, dendritic cells, placental trophoblasts, and epithelial cells. *Brucella* species can survive under extreme conditions of temperature, humidity, pH, and persist in frozen and aborted materials for longer durations [6].

Although several countries are affected by brucellosis, it is still a neglected disease and no official program for surveillance and eradication of animal brucellosis has been proposed [7]. In many developing countries of Africa, Asia, Central, and South America, clinical disease is recorded among different animals [8, 9]. The impact of brucellosis on human health is a major issue [10]. In humans, it causes fever, nausea, muscular pain, abdominal pain, sweating, weakness, decreased appetite, weight loss, and liver inflammation [11]. This disease in domestic animals poses a threat to free animal movement and trade of animals and various animal products and causes huge economic losses. It also leads to economic burden due to decreased milk production, breeding failure, and abortion in the domestic animals infected with *Brucella* spp. [12].

The development of DNA markers and molecular biology techniques are important tools for genomics-based studies in animal biotechnology [13]. It has led to genetic improvement and markers now help in the selection of best quality breeds of animals. Over the last 20 years, DNA markers have resulted in tremendous genetic improvement in farm animals. Recent developments in molecular biology techniques have revealed genetic polymorphisms in DNA sequences, which have been used extensively as markers for assessing the genetic basis of the phenotypic variations in animals. The genetic markers indicative of changes at the DNA level are called molecular markers [14]. The PCR has become an important tool for molecular DNA studies, including the detection of DNA polymorphism (fingerprinting), analysis of genotyping, and genome

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mapping. Along with microbial techniques, 16S rRNA gene sequencing and RAPD methods are very essential in molecular diagnosis. Using an array of random single RAPD primers, it is possible to determine the polymorphism that can be used in DNA analysis [15].

Improvement of the livestock industry and the use of improved prevention measures to control brucellosis, require an understanding of the disease risk and the prevalence of infection in the human population and animal hosts. Epidemiological studies of brucellosis in humans as well as domestic animals globally is urgently needed to effectively control this disease [16–21]. The objective of this work are to describes the current status of brucellosis and the risk factors of the disease in animals and the human population. In addition, there is a further discussion of the various issues related to the control and prevention of brucellosis in domestic animals and humans.

2. Historical background of Brucellosis

The disease brucellosis has existed since time immemorial. The analysis of the skeleton of *Australopithecus africanus* from the late Pliocene era revealed the effect of brucellosis in human ancestors [15]. Furthermore, DNA analysis and pathological findings from human skeletal remains and buried cheese remains indicated the presence of brucellosis in some countries in the Middle East and Europe in 79 A. D. [15, 16]. The earliest record of this disease was in 1859, and it mentioned that animal abortions were most probably due to brucellosis [1]. However, in the 1880s, the causative agent of this disease, *Brucella melitensis*, was isolated and identified. The term brucellosis was named after Sir David Bruce who isolated and characterized the infectious agent from a soldier in Malta in 1886. This disease was responsible for inflicting severe mortality and morbidity in British military personnel in Malta and hence was also well-known as Malta or Mediterranean fever [6]. It was also known as Bang's disease after the isolation of the causative agent *Brucella abortus* in 1897 by Danish veterinarian Bernhard Bang. Due to the "wave-like" characteristic of the fever, which rises and falls over several weeks in patients, it is also known as "undulant fever". Other names for brucellosis include Gibraltar fever, Rock fever, Cyprus fever, and typhomalarial fever in humans and animals.

3. Prevalence of brucellosis

Although Brucellosis has been very well controlled in many developed countries, it is still a major concern in Asia including Middle Eastern countries, Africa, Mediterranean countries and South America. Human brucellosis has re-emerged in China since 1990, due to the drastic growth of animal husbandry which increases the probability of human infection [3]. Brucellosis is still a major concern in the Indian subcontinent. India has one of the largest bovine populations in the world, which is responsible for the continued exposure of workers in milk industry to these animals and hence there are high incidences of brucellosis in humans [17]. A seroprevalence study in India indicated brucellosis seroprevalence ranging from 2% to 18% in suspected patients [17]. Several countries which were endemic such as Malta, France, Ireland, Israel have been successful in eradicating the disease. Studies in various countries, have indicated that *B. melitensis* is the main cause of human brucellosis, while infection with *B. abortus* is less frequent [22]. Domestic animals are a natural reservoir of *Brucella* spp. and animal-to-human transmission occurs through the consumption of infected meat and milk. Serological data for the prevalence of brucellosis in various provinces in Saudi Arabia is still not available [22, 23]. The incidence of the disease had been reported from the Northern, Southern, and Central regions of Saudi Arabia [24, 25]. A seroprevalence study by Ageely and colleagues in the Jazan province of Saudi Arabia revealed that the prevalence of human brucellosis was higher in patients ≥ 40 years old (20 %) as compared to the population < 40 years (12 %). Seroprevalence was higher in the rural population (39.3%) than in the urban population (4.6%), and significantly higher in the Saudi population (14.5%) compared to the non-Saudi population (3.0%). However, the prevalence was much higher in males (16.4%) than females (7.1%) [16]. *Brucella* spp. infection in humans is mainly through the consumption of raw milk and meat products derived from infected goats or camels. It has also been observed that laboratory workers, hospital staff, and veterinarians are more prone to brucellosis [23, 26, 27]. Brucellosis is caused by bacteria belonging to genus *Brucella* in humans and several other animals, including goat, cows, buffaloes, sheep, pigs, camels and reindeer [1, 28].

4. Taxonomy

Brucella spp. are Gram-negative cocci or small rods, non-motile, non-encapsulated, non-flagellated, non-spore forming aerobic microbes. It has the capability to invade, epithelial cells, macrophages, dendritic cells and placental trophoblasts [7].

Genus *Brucella* belongs to the alpha-2 subdivision of class *Proteobacteria* and 10 different species of *Brucella* based on the host specificity that has been reported. These species are *B. melitensis* (goats), *B. abortus* (cattle), *B. ovis* (sheep), *B. canis* (dogs), *B. neotomae* (desert woodrats), *B. suis* (swine), *B. pinnipedia* (seal), *B. microti* (voles), *B. cetacea* (cetacean), and

B. inopinata (unknown) [29]. Among the known 10 species of *Brucella* only *B. melitensis*, *B. abortus*, *B. canis* and *B. suis*, have been found to cause infection in humans. *B. ovis* and *B. neotomae* are not pathogenic to humans. Most of the human infection cases globally are caused by *B. melitensis* [30]. *B. melitensis* and *B. suis* are more infectious and virulent in humans compared to *B. canis* or *B. abortus* [31]. *B. melitensis*, *B. suis* and *B. abortus* are known to have 3, 5, and 7 subtypes, respectively [32, 33]. Sequencing the genome of *Brucella* species revealed a sequence homology of more than 99% among the species [29, 34].

Most *Brucella* species possess smooth lipopolysaccharide (SLPS) in the outer cell wall, while *B. canis* and *B. ovis* have rough lipopolysaccharide (RLPS) and protein antigens [35]. SLPS contains an immunogenic O-polysaccharide, defined as a homopolymer of 4, 6-dideoxy-4-formamide-α-D mannose, which is connected by glycosidic linkages. *Brucella* species that possess smooth lipopolysaccharide, particularly *B. melitensis*, are known to undergo dramatic structural variations during growth, and more often change to rough form (R) and occasionally to mucoid form (M). During the change in morphology to rough form (R), bacterial colonies appear to be less transparent, with a more granular surface. During the change in morphology to mucoid form (M), the bacterial cells appear to have a gelatinous texture and their color changes from white to brown in reflected light. An intermediate form (I) has also been observed during the change in morphology from smooth (S) to rough form (R). It had been shown that changes in the bacterial cell morphology are associated with marked differences in virulence, pathogenicity and serological properties of *Brucella* spp. [36].

5. Epidemiology of Brucellosis

The epidemiology of brucellosis has dramatically changed over the past few years due to improvement in hygiene, socio-economic conditions and an increase in international travel. Incidence of human brucellosis has been reported for the first time from the regions of central Asia and in some countries. Particularly in Middle East countries, there is a drastic increase in the incidences of brucellosis in humans [30].

Brucellosis affects domestic as well as wild animals. It has been reported to occur worldwide wherever animals are raised [37]. Although some industrialized countries in Europe and America have been able to eradicate brucellosis in domestic animals through intensive control schemes, the disease is still a severe problem in several developing countries [38].

B. melitensis is the most pathogenic species and comprises three biovars. Biovars 1 and 3 have been detected most often in domestic animals in the Middle-East, Mediterranean and Latin American countries [39, 40]. Brucellosis is considered a major barrier to the free trade of domestic animals and various animal products and is responsible for significant economic losses due to abortion in domestic animals [41].

6. Risk factors for brucellosis

Various factors such as environmental factors and the host biology can affect the occurrence and prevalence of brucellosis. Some of these factors include the age of the animals, herd size, sanitary conditions of animal farms and climatic conditions [24, 42] It has also been observed that sexually mature animals are more prone to the infection and bacteria mainly localize in the reproductive organs, particularly in pregnant animals. Besides, *Brucella* spp. may also be localized in the mammary glands [43].

Many people in poor African countries such as Ethiopia are dependent on livestock for their livelihood which leads to their close association with the domestic animals, increasing the risk of infection through *Brucella* spp. [42, 44]. Cases of brucellosis are very high in rural areas as farmers live closely with their domestic animals and more often consume unpasteurized milk products [24]. In countries like Ethiopia, the habit of consuming raw milk, improper handling of an aborted fetus and reproductive excretions are responsible for the transmission of zoonotic diseases like brucellosis to humans [45].

In many other countries, risk factors for infection with *Brucella* include consumption of contaminated animal products such as milk and meat, handling of infected animals, traveling to an endemic area and mishandling cultures of *Brucella* sp. in laboratories and diagnostic centers. Veterinarians, dairy workers, and slaughterhouse workers are more susceptible to infection with *Brucella* [8]. Recent studies showed a poor community's knowledge of brucellosis and the risk associated with brucellosis among people living adjacent to Awash National Park in Ethiopia [46]. Hence, there is an utmost need to create awareness about the disease, improve knowledge, attitudes and practices among livestock owners, which would further lead to a significant reduction in the transmission of the bacteria from animals to humans in the disease-prone areas [41].

7. Transmission

Brucella melitensis has been found to be sexually transmitted in sheep and goats. The transmission of brucellosis is facilitated by the intermingling of animal herds that belong to different owners and by the procurement of cattle from sources that are not properly screened for the disease [2]. Moreover, using common male breeding stock also increases the risk of disease transmission among domestic animals. Other factors that can promote the transfer of infection include the intermingling of animals during grazing, the crowding of animals in farms, marketplaces or animal fairs [1]. Following an abortion in domestic animals, animal farms can become contaminated with *Brucella* spp. and other animals on the farm may acquire the disease by ingestion, inhalation, skin contamination or udder inoculation. Sometimes, pooled colostrums used for feeding newborns can also play a role in the transmission of the disease. Artificial insemination may also promote the transmission of the *Brucella* spp. to healthy animals.

In humans, brucellosis is caused by *B. melitensis*, *B. abortus* and *B. suis* which are transmitted by an infected goat, pigs, sheep or cows to healthy humans [47]. Besides, domestic and wild animals are also infected with *Brucella* sp. and can act as reservoirs of bacteria that can be transmitted to both humans and domestic animals [41]. Exposure of humans to infected domestic animals or the consumption of milk or meat products derived from infected animals enhances the risk of acquiring brucellosis [8]. The transmission of brucellosis may also occur through blood transfusion or organ transplantation. Some people such as farmers, farm laborers, animal attendants, shepherds, and veterinarians are at a very high risk of acquiring the infection. These workers are always at a very high risk of infection with *Brucella* spp. due to direct contact with infected animals or constant exposure to the contaminated environments. The main source of *Brucella* infection in the urban population is usually contaminated food, milk or dairy products derived from infected animals [20, 47].

8. Clinical Symptoms

Animals

The incubation period in brucellosis is found to be highly variable and is defined as the period between the exposure and first appearance of clinical symptoms or abortion. In cows, infected at the early stage of pregnancy, abortion may occur after 225 days. But for those infected at seven months gestation, abortion may occur after 50 days. Various factors such as age, sex, stage of gestation, infective dose and immune system of the animals may influence the incubation period [2, 48]. In very susceptible animals with weak immune systems, abortion occurs during the third trimester and other clinical symptoms include metritis, retained placenta, and reduced milk production [49]. It has been observed that abortion may occur in 80% of animals that are infected with *B. abortus* [48]. Brucellosis is one of the major causes of infertility in camels, cows, goats, sheep, pigs, and dogs [50, 51]. Infection in male animals causes hygromas, orchitis, and inflammation of the seminal vesicles [50].

9. Human brucellosis

In humans, there are three stages of brucellosis i.e. acute, subacute or chronic phase and the incubation period is two to three weeks and sometimes several months. The predominant symptoms of brucellosis in humans include intermittent fever, headache, backache, weakness, weight loss, anorexia and mental depression [52, 53]. During the chronic phase, knee joints are also affected [54]. Complications may occur in the gastrointestinal, cardiovascular, pulmonary, lymphatic, and nervous systems [55, 56]. The effect of *Brucella* infection on the nervous system causes neuro-brucellosis, which is characterized by fever, headache, psychosis, seizures, behavioral changes, and spastic paresis [57].

10. Diagnosis of brucellosis

It has always been difficult to clinically diagnose brucellosis in animals as well as in humans [13]. Presently, the serological method, culture-based method, and molecular techniques are employed to detect *Brucella* infection in animals and humans [58]. Diagnostic tests are based on the detection of the causative agent *Brucella* sp., the detection of antibodies in the serum and allergic reactions [59].

11. Detection of *Brucella* organisms

Culture

Culture-based methods are employed to detect *Brucella* spp. in milk, blood or colostrum, including fetal membranes, uterine discharges of infected animals and aborted fetuses. The supra mammary lymph nodes or retropharyngeal or prescapular lymph nodes are also very suitable samples for the diagnosis of brucellosis in animals [48, 60]. For the diagnosis of brucellosis in humans, blood, urine and cerebrospinal fluid are screened in order to detect the bacteria, particularly during the acute stage of brucellosis [12, 14, 61]. However, culture-based methods are not suitable for the diagnosis of brucellosis during the chronic phase, as the bacterial count is very low. Another major drawback of the culture-based methods is the slow growth rate of the *Brucella* species. Moreover, there is a very high risk to the health of the laboratory personnel [1, 12, 13].

Microscopic examination

Staining methods such as Ziehl Nelsen staining can also be used to detect *Brucella* in infected animals. However, this staining technique is not very specific to *Brucella* spp., as other microorganisms such as *Chlamydia*, *Coxiella*, and *Nocardia* exhibit acid-fast features [47].

12. Serological tests

Several serological tests are currently being employed for qualitative and quantitative detection of specific immunoglobulins of *Brucella* organism-specific antibody titer in the infected animals. Serological tests such as Rose Bengal Plate test (RBPT), Complement Fixation Test (CFT), Serum Agglutination Test (SAT), and Enzyme-Linked Immunosorbent Assay (ELISA) are routinely used for diagnosis of brucellosis in animals and human [60]. Milk Ring Test (MRT) is employed for the detection of *Brucella* organisms in infected animals [62].

Serum Agglutination Test (SAT)

Globally, this test has been used for the detection of brucellosis in infected animals and humans for decades [63]. However, there are some drawbacks associated with this technique, which limit its utility. This test is unable to distinguish natural *Brucella* infections from the vaccination effect. It is also unable to detect *Brucella*-specific antibodies after abortion in infected animals and during the chronic phase of brucellosis [48, 50].

Rose Bengal Plate test (RBPT)

Rose Bengal test is usually used for the diagnosis of brucellosis in most countries. It has been effectively used for screening domestic animals, wildlife and the human population [64]. The probability of obtaining false-negative data is very rare. However, there is a possibility of getting false-negative results during the early stages of infection, whereas false-positive results can occur due to vaccination and cross-reactivity [48].

Milk Ring Test (MRT)

This test is used for the screening of domestic animals to detect brucellosis infection [48]. However, the major limitation of this test is its very low sensitivity compared to other techniques such as ELISA [65]. The comparison of the MRT and ELISA techniques by testing milk and sera, respectively, obtained from the same female animals indicate that the ELISA test revealed more positive animals as compared to MRT [59]. Low sensitivity of the Milk ring test is attributed to mastitis, vaccination with S19, temperature variations and the use of soured milk in the test [48].

Complement Fixation Test (CFT)

The major advantage of the complement fixation test is its high specificity and sensitivity and it is a commonly used test for serological detection of brucellosis infection in domestic animals and humans [66]. Non-specific reactions are not an issue in the Complement Fixation Test. Moreover, unlike the Serum agglutination test, the CFT is more suitable for screening brucellosis infection during the chronic stage of the disease.

Enzyme Linked – Immunosorbent Assay (ELISA)

The diagnosis of *Brucella* infection has improved through the development of ELISA technology [55, 65]. Among various ELISA methods, the Competitive ELISA (c-ELISA) is more robust, very sensitive, and highly specific. The c-ELISA has the capability to differentiate naturally infected animals from the vaccinated ones and also has the ability to rule out animals infected with cross-reacting microorganisms. The c-ELISA can be performed by using the serum as well as milk samples from different animal species without compromising the sensitivity and specificity [64, 67].

13. Molecular diagnostic methods

Currently, molecular biology techniques are being used extensively to identify the causative agents, as these techniques are less time-consuming, have high specificity and sensitivity to detect microorganisms [60, 68]. In addition, restriction endonuclease and hybridization have been used extensively for decades for *Brucella* detection [14, 69].

PCR is very sensitive, highly specific, rapid, and easily amenable for high-throughput screening. It is also more suitable for the detection of slow-growing bacteria such as *Brucella* [70]. Due to its very high sensitivity, the PCR based method has the capability to detect tiny amounts of bacteria in clinical samples. It has also been demonstrated that the PCR technique is able to detect dead microbes in clinical samples, hence reducing the need for proper sample preservation before analysis [71]. This method of detecting *Brucella* infection is highly reproducible and very reliable, however, care should be taken to avoid contamination during analysis in the laboratory [72].

PCR-based protocols for the detection of *Brucella* spp. in clinical samples have been designed and developed. These methods are based on the amplification of gene BCSP31 which is highly conserved among *Brucella* spp., or the amplification of the 16S rRNA gene [72]. This approach is useful for screening when biovar or species designation is not critical. In order to distinguish *Brucella* species or biovar, PCR protocols have been developed based on gene loci, which vary among biovars and species. However, such gene targets are not common in *Brucella*, as the genus is unusually homogeneous and conserved. While few deletions and rearrangements have been found within a biovar or species, most of the differences at genetic levels consist of single nucleotide polymorphisms [69]. Differential PCR based methods are of great value for epidemiological studies, disease monitoring, and species-specific eradication program. The differential PCR protocols can be denoted as species-specific and genus specific. In the case of species-specific methods, three approaches have been reported (i) protocols designed with highly specific primers and stringent assay conditions; (ii) protocols developed with low-specific primers and less stringent PCR conditions, and (iii) protocols designed with random primers under very flexible assay conditions [73, 77].

14. Control and prevention of brucellosis

Brucellosis is an infectious disease that has been controlled and eradicated in some countries in the world [3]. In sub-Saharan Africa, animal health services have been substantially deteriorated over the last 20 years due to various factors such as reduction in government budgets, especially the funds required to control brucellosis [81] Hence, various programs that require the use of disease prevention measures, information exchange, and coordinated surveillance, are not properly implemented in many sub-Saharan countries [2, 18, 42, 82].

The primary objectives for the control and prevention of brucellosis are centered on the economic impacts of the disease and its public health consequences [73]. Control activities mainly reported by countries include surveillance, controlling the movement of domestic animals, treatment of meat and milk products and animal vaccination, [3, 42, 84]. In Mozambique, the control of brucellosis was well organized by using the S19 vaccine in cattle until 1980. A strain 19 vaccine produced by the Underreport Veterinary Institute in South Africa containing viable *Brucella* cells was used. The vaccine was administered subcutaneously to heifer calves at four to eight months of age. However, recently some private farmers have been using S19 vaccination in adult cows. Also, surveillance and movement control were also implemented at a very low level, resulting in a drastic increase in cases of brucellosis. *Brucella abortus* vaccine, strain RB-51, live culture, licensed in 1996, has been extensively used in USA to eradicate brucellosis [73–77].

Antibodies against *Brucella* cell wall O-polysaccharide (OPS) component of smooth lipopolysaccharide is known to confer protective efficacy to the currently used vaccines. However, the detection of these antibodies is also used in diagnosis of brucellosis in animals, and so it becomes very difficult to differentiate vaccinated animals from infected animals and this hampers the effort to control the disease [75]. In order to sort out this issue, it has been proposed that combining anti-*Brucella* OPS antibody response with the induction of cell-mediated response would provide highly effective protection against brucellosis which can be achieved by the conjugation of O-polysaccharide (OPS) to a highly immunogenic *Brucella* protein. Such type of glycoconjugate vaccine would be more effective in protecting animals and humans against brucellosis and would not interfere with the diagnostic testing for *Brucella* infection [75].

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