

Review Article

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Brucellosis: A Disease of Zoonotic Importance

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ABSTRACT

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Brucellosis is one of the important bacterial zoonotic diseases worldwide. It is caused by the Gram-negative intracellular coccobacillus *Brucella*. Till today, the brucellosis remains as essential threat to animals and humans, particularly in developing areas of the world. *Brucella* spp. infects a variety of domestic and wild animals. In humans, it affects people of all age groups and of both sexes. Transmitted by direct or indirect contact with infected animals or their products. The disease is suspected to be the cause of abortions which remain largely undiagnosed in both humans and animals.

Introduction

Brucellosis is a zoonosis of both veterinary and public health significance with an economic impact on livestock production in most of the developing countries like India (Corbel, 1997; Tarfarosh and Manzoor, 2016). According to OIE, it is the second most important zoonotic disease in the world after rabies with the annual incidence of half a million cases globally (Golshan and Buozari, 2017). Brucellosis has been identified as one of the most significant neglected zoonotic diseases in the world (Corbel, 1997; Franc *et*

al., 2018). The disease is one of the most devastating transboundary animal diseases and also a major trade barrier. It is caused by *Brucella* species which are small, Gram-negative and coccobacilli bacteria. The disease affects wide range of domestic and wild animals as primary hosts and humans as secondary hosts (McDermott *et al.*, 2013; Tali *et al.*, 2015). Brucellosis was first recognized as a disease affecting human-beings on the island of Malta in the 19th and early 20th centuries. The bacteria are transmitted from animals to human by ingestion of infected food products (meat or raw milk), direct

contact with infected animals or their tissues or inhalation of aerosols. Humans are accidental hosts, but brucellosis continues to be a major public health and zoonotic concern worldwide (CDC, 2012; Giambartolomei and Delpino, 2019).

It is a chronic granulomatous infection, capable of affecting any organ system (Pappas *et al.*, 2005). Brucellosis also generates significant economic impact by causing serious production losses through abortions, infertility and decreased milk production in cattle, goats, sheep, swine and camels (McLeod, 2011). In humans the symptoms varies from an acute, non specific febrile illness to chronic, debilitating forms with features of osteo-articular and neuropsychiatric abnormalities (Rubach *et al.*, 2013). Bacterial isolation is considered as the “gold- standard” for specific diagnosis of *Brucella* spp. (OIE, 2016). The molecular and serological tests such as, Polymerase Chain reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA), Milk Ring Test (MRT) and the Rose Bengal Test (RBT) can be used to diagnose brucellosis. Measures against brucellosis should aim at the control and if possible, the eradication of the agent in the animal reservoir. The focus should be on preventive measures such as vaccination of livestock coupled with strengthening the curative health care services for early diagnosis and treatment. This review highlights the etiology, transmission, methods for early diagnosis, treatment and prevention and control measures.

Etiology

Brucellosis was first described in 1887 by David Bruce, a British surgeon, who isolated Gram-negative coccobacilli from the spleens of five British soldiers who died of fever in Malta (Bakri *et al.*, 2018) which was known as Malta fever and it was common among

military personnel stationed on the island of Malta. The bacterium was named *Micrococcus melitensis*, with ‘melitensis’ derived from the Roman name for Malta, ‘Melita’. In 1897, *Bacillus abortus* was identified as the cause of contagious abortion in cattle by Bernhard Bang. Later, in 1917, it was found that the causes of the two diseases were identical, and renamed *Brucella* in honor of Bruce (Perkins *et al.*, 2010).

At present, 12 species of *Brucella* genus have been described of them six are known to be pathogenic for both animals and humans (Eisenberg *et al.*, 2012; Ntirandekura *et al.*, 2018). *B. abortus* preferentially infects cattle, *B. melitensis* sheep and goats, *B. suis* pigs and *B. canis* dogs (Mantur and Amarnath, 2008) while the other members include *B. ovis* and *B. neotomae* (Corbel, 1997). Cross transmission of brucellosis can occur between cattle, swine, sheep, goats and other species including dogs, horses, bison, rein deer and camels (FAO, 2003).

Brucella melitensis causes the majority of cases globally and has a predisposition for recurrence and chronic stages. They are Gram-negative, partially acid fast, aerobic, facultative intracellular coccobacilli or short rods. They are oxidase, catalase, nitrate reductase and urease positive. The cells are short and slender; the axis is straight; the ends are rounded; the sides may be parallel or convex outwards. In length they vary from about 0.5 - 0.7 μm , in breadth vary from 0.5 - 1.5 μm , occurring singly, in pairs or short chains (Mantur and Amarnath, 2008). The presence of rough or smooth lipopolysaccharide correlates with the virulence of the disease and smooth are generally more virulent. *Brucella* species and their different biotypes are currently distinguished by differential tests based on serotyping, phage typing, dye sensitivity, CO₂ requirement, H₂S production and metabolic properties.

Transmission and risk factors

In animals

The main mode of transmission in animals includes ingestion but transmission can also occur by inhalation of infected aerosol, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups. Aborted fetuses as well as fetal membranes and uterine secretions eliminated after abortion or parturition are the most important sources of infection (Samartino and Enright, 1993). Animals get the infection by ingestion of contaminated feed and water, licking of aborted materials and fetus and vagina of infected animals. The disease can also be transmitted to calves vertically and through contaminated milk but these routes of infection are much less important (Nicoletti, 1980). Venereal transmission is rarely involved in the epidemiology of bovine brucellosis however; artificial insemination with contaminated semen is a potential source of infection (WHO, 2006).

In humans

Humans are accidental hosts, but brucellosis continues to be a major public health concern worldwide. The incidence of human brucellosis depends on some of the aspects like, husbandry practices, dietary habits, methods of processing milk, and dairy products, as well as environmental sanitation. Humans acquire the infection from infected animals by means of ingestion (unpasteurized milk or dairy products), inhalation of aerosols, conjunctiva or by contact with infected animal and their products (Avila-Calderon *et al.*, 2013; Bosilkovski *et al.*, 2015). Brucellosis occurs mainly in slaughter house workers, veterinarians, lab technicians, hunters, farmers and livestock producers because they come into direct contact with infected animals and tissues from those

animals. Human to human transmission is very rare however it may be transmitted via blood transfusion, bone marrow transplantation, sexual contact or congenital (WHO, 2006; Gwida *et al.*, 2010). Veterinarians may get the infection while supporting births in infected livestock and unintentional vaccine exposure (Malak Al Anazi *et al.*, 2019).

Symptoms

In animals

The disease in animals is also known as enzootic abortion, epizootic abortion, contagious abortion and Bang's disease. It is a sub-acute or chronic disease which may affect a wide range of domestic and wild animals. In the initial phase of infection the disease remains asymptomatic (WHO, 2006). In livestock, brucellosis decreases productivity by causing abortions, reducing fertility and decreasing milk yield (Corbel, 1997; Alsaif *et al.*, 2018). The disease is manifested by late term abortions, weak calves, still births, infertility and characterized mainly by placentitis, epididymitis and orchitis with excretion of the organisms in uterine discharges and milk (England *et al.*, 2004; Abubakar *et al.*, 2012). Infected bulls may develop systemic signs of infection including fever, anorexia and depression. Brucellosis during the course of pregnancy carries the risk of spontaneous abortion or intrauterine transmission to the infant.

In humans

The disease in humans is also known as undulant fever, Malta fever and Mediterranean fever. In humans, brucellosis involves multiorgans with a complicated and various clinical presentations ranging from non-specific to severe symptoms (Ulu-Kilic *et al.*, 2013), which makes brucellosis easily

misdiagnosed as other diseases. Based on the course of the disease, human brucellosis is classified into three forms: (1) acute brucellosis characterized by weakness, undulant fever, headaches, myalgia, fine red rash, splenomegaly, hepatomegaly and gastrointestinal symptoms.

The acute phase may end in death, curing, transition into a sub-acute or chronic form; (2) sub-acute brucellosis characterized by almost all symptoms typical of the acute course but milder; (3) chronic brucellosis in which long-term signs and symptoms may include sweats, fatigue, undulant fever, arthritis, endocarditis and spondylitis, personality changes (Galinska and Zagórski, 2013; Moosazadeh *et al.*, 2016). It is often misdiagnosed as other febrile syndromes, such as malaria and typhoid fever, resulting in mistreatments and underreporting (Halliday *et al.*, 2015).

Diagnosis

The diagnosis of brucellosis involves the consideration of medical history, clinical evaluation and routine laboratory and radiologic tests combined with culture, serology or polymerase chain reaction (PCR) assay (Kurdoglu *et al.*, 2015). The gold standard specific test for diagnosis of *Brucella* spp. is bacterial isolation but it has some disadvantages that it is time-consuming and requires biosafety level 3 and expert personnels (Alton *et al.*, 1988; OIE, 2016).

Molecular approaches are rapid and convenient for the diagnosis of *Brucella* spp. from serum, blood, pus and tissue. Now a day's real-time quantitative PCR (qPCR) appeared to be highly reliable to assess the occurrence of *Brucella* contamination in milk and may enable the discrimination of virulent strains from those resulting from vaccination (Awwad *et al.*, 2016).

The conventional and most available serological tests for the detection of particular antibodies against *Brucella* antigens in milk and serum includes the Milk Ring Test (MRT), Rose Bengal Test (RBT), Standard Tube Agglutination test (SAT), Coombs test, Complement Fixation test. The Rose Bengal test can be employed as a screening test and positive samples are confirmed by the SAT. The sensitivity of the Rose Bengal plate test is more than 99%. The SAT remains the most accepted diagnostic test worldwide. SAT measures the total quantity of agglutinating antibodies (IgM and IgG) and the quantity of specific IgG is determined by 2-mercaptoethanol (2ME). SAT titres above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation. In endemic areas, a titre of 1:320 as cutoff may make the test more specific (Mantur and Amarnath, 2008). The complement fixation test (CFT) is commonly used for the diagnosis of brucellosis in cattle, sheep and goat. It detects mainly IgG1 antibodies but this test is somewhat complex and it cannot differentiate infected animals from vaccinated ones (Staak *et al.*, 2001). Enzyme-linked immune-sorbent assay (ELISA) that measures IgG, IgM and IgA antibodies has advantages of having high sensitivity and possibility of better interpretation of the clinical situation. However, the specificity of ELISA is less in comparison to the agglutination tests (Christopher *et al.*, 2010). Coomb's test is the most suitable and sensitive test for confirmation in relapsing patients with persisting disease, but it is complex and demands technique (Smith and Kadri, 2005). The lateral flow assay (LFA), a simplified version of ELISA has a great potential as a rapid point-of-care assay. This test has high sensitivity and specificity. It is a rapid and simple diagnostic test for confirmation of brucellosis in an endemic area (Hasanjani *et al.*, 2005).

Treatment

In spite of the application of WHO's antibiotic regimen recommendation (1986), which consists of doxycycline 100 mg orally twice a day for 6 weeks plus oral rifampicin 600 to 900 mg daily for 6 weeks or streptomycin 1g intramuscularly daily for 2-3 weeks, the rate of brucellosis treatment failure and relapse has been increased by 5-15%. The choice therapeutic regimen for the uncomplicated brucellosis consists of streptomycin for 2 to 3 weeks plus doxycycline for 8 weeks or gentamicin for 5-7 days plus doxycycline for 8 weeks (Ranjbar, 2015). The second-line agents such as quinolones or trimethoprim-sulfamethoxazole can be administered for patients with treatment failure or repeated relapses. For patients with a complicated disease, treatment intervention requires a careful evaluation of the patient and a thorough therapeutic plan. Patients with spondylitis should possibly receive a quinolone in the initial regimen, for a protracted period (Alavi and Alavi, 2013).

Prevention and control

Prevention and control of brucellosis in humans largely depends on successful control of the disease in livestock. In 1998, WHO recommended common strategies for the eradication of animal brucellosis. The strategies and program included (1) prevention of disease extension among animals and monitoring brucellosis-free herds and regions (2) identification of infected animals using diagnostic tests and their elimination by slaughter programs to generate brucellosis-free herds and zones and (3) applying vast vaccination programs to decrease the disease prevalence.

In endemic areas, pasteurization of milk and milk products is considered as a significant

safety method. Consumption of unpasteurized milk and dairy products and also raw or undercooked animal products (including bone marrow) must be avoided. Occupational exposure to *Brucella* can be prevented by good hygiene and using protective clothing/equipment. The use of safety measures are essential to prevent skin contamination, inhalation, or accidental ingestion of organisms while assisting at the birth, carrying out a necropsy or butchering an animal. Moreover, handling an aborted fetus or its membranes and fluids requires a special precaution. (Avila-Calderon *et al.*, 2013). Thus, improved veterinary services and public health education may play an important role in the disease control (Alavi *et al.*, 2014). These goals have been achieved in some countries by use of vaccination, test and slaughter policy and strict control of animal movement (Tahmoorespur *et al.*, 2016).

Globally, vaccination has extensively contributed to the prevention and eradication of brucellosis. Vaccination is important to improve the health of animals and also an important step to reduce the risk of severe illness and disability. Vaccination against brucellosis is practiced in countries with high prevalence of more than 5%, particularly in developing countries since vaccination is relatively cheap and readily acceptable by the farmers (Blasco and Molina-Flores, 2011). The current available vaccines for both bovine and caprine brucellosis contain live attenuated organisms. Cattle vaccines contain either the smooth strain *B. abortus* S-19 or the rough strain RB-51 (Kumar *et al.*, 2016; Ebrahimpour, 2015) while caprine vaccine contains attenuated *B. melitensis* vaccine strain Rev-1 (Avila-Calderon *et al.*, 2016). These live attenuated vaccines were shown to be effective in preventing abortion and transmission of brucellosis, but poor at preventing infection or sero-conversion (Olsen *et al.*, 2013). Educational programs

targeting at risk populations, along with stringently enforced hygiene measures, regulations and inspections, should be implemented to reduce the incidence of this disease in brucellosis endemic regions (Dadar *et al.*, 2019).

In conclusion, brucellosis is a zoonotic disease which is endemic in low, middle and high-income countries that causes devastating losses to the livestock industry. It places significant burdens on human healthcare systems and limits the economic potential of individuals, communities and nations. The implementation of public policy focused on mitigating the socioeconomic effects of brucellosis in human and animal populations is desperately needed. The interdisciplinary “One Health” effort which indicates the collaborative approach of veterinary, medical, public health, cultural, economic and social experts is needed reduce the burden of brucellosis.

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