https://doi.org/10.47278/book.vpph/2021.026

PATHOGEN, HOST CELL RESPONSE, DIAGNOSIS AND THERAPY OF BRUCELLOSIS

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INTRODUCTION

Brucellosis is one of the most ubiquitous zoonosis with global distribution (Gul et al. 2015; Massis et al. 2019). Etiology of this disease is Brucella that is a facultative intracellular pathogen. In Brucella genus, 11 species are recognized (Gul et al. 2013; Mesureur et al. 2018). The disease may affect bovine, caprine, ovine, swine, and humans. Due to lack of hygienic processes, public health measures, even national animal health managing policies, the disease is more common in developing countries (Thakur et al. 2002; Farouk et al. 2017; Hasan et al. 2021). Brucellosis causes abortion, reduced fertility, decreased milk production and cost of replacement (Khan and Zahoor 2018). Serious socioeconomic issues can be posed by the disease to livestock owners (Khan et al. 2020). Due to rapidly increasing intercontinental tourism and animal trade, there are high chances that the disease could spread in developed countries (Imtiaz et al. 2018). Brucellosis in humans frequently results in a typical undulant fever, with osteoarthritis as usual impediments (López-Santiago et al. 2019). The intracellular biology of the Brucella is the consequence of complicated interfaces with host that is mandatory to determine a role of pathogen existence and multiplication. In spite of the risk to the public health, there are no effective vaccines to counteract many of them. In this chapter, advances in the field of the pathogen, host cell response, diagnosis and therapy of brucellosis are described.

History, Spread and Pathogen

Huge economic losses are rendered by the Brucellosis (Shahzad et al. 2018), especially in food animal production sector. The economic losses in animals due to Brucellosis are primarily due to abortions, occurring during the last trimester, decreased milk yield, transient infertility and perinatal mortalities (Gul et al. 2015; Zeng et al. 2019; Khan et al. 2020). The disease is endemic in the buffalo and cattle, causing approximate economic loss of US $ 344 billion to the animal industry (Pal et al. 2020). In some countries of the world, animal Brucellosis has been eradicated, but in many other countries it remained uncontrolled (Gomez et al. 2013). Due to its zoonotic aspect, high incidence in humans has been reported from different countries (Wang and Jiang 2020), including Yemen (89.96%), Kenya (203.07 cases per 100 000), Syria (47.26%), Greece (42.96%) and Eritrea (21.82%). However, this disease continues to exist, particularly in Africa, India, South and Central America, Middle East and the Mediterranean region (Baldane et al. 2012; Wang and Jiang 2020; Akya et al. 2020). In the past, in humans, 0.5 million new cases have been reported due to Brucellosis annually (Franco et al. 2007; Pal et al. 2020). On the basis of published reports about Brucellosis, Iran stands second in the world with an annual prevalence of 98-130 people/100,000 populations (Marvi et al. 2018).

It is documented that David Bruce from Royal British Medical Staff examined it as “Malta fever” or “Mediterranean fever”, while the British troops were enduring high fever for long time. David Bruce was able to culture the bacterium liable for the disease in 1887. Afterwards, Themistocles Zammit’s discovered that people rearing goats, and drinking their milk also exhibited similar signs, as those of Mediterranean fever patients. Then, Zammit was able to reproduce the disease in healthy goats, who also isolated Brucella from milk and blood. He concluded that the personals of British Army could have caught the disease by drinking milk from contaminated goats (López-Santiago et al. 2019). Based on these results, though, a decision to ban goat’s milk consumption in the British army was made in 1906, however, Malta fever was not eradicated but doubts evolved regarding the use of cheese, and ice-cream made from contaminated milk. Findings of Zammit demonstrated that Brucellosis is commonly transmitted via oral route. Later, other routes were documented (parenteral, respiratory, or by contact) and the disease was believed to be the occupational hazard (Mantur et al. 2007; Gomez et al. 2013; Gul et al. 2015; Shahzad et al. 2017). It has been reported that following possible risk factors can be responsible for human brucellosis: i) eating contaminated animal products, ii) occupational exposure, and iii) contact with diseased animals or their products and/or discharges (Pal et al. 2020).

Classification of Brucella spp. is established on host inclination and virulence (Cloeckaert et al. 2002). The genus Brucella consists of seven species based on primary host and antigenic variation: *Brucella abortus* (cattle), *B. melitensis* (sheep and goats), *B. ovis* (sheep), *B. canis* (dogs), *B. neotomae* (wood rats), and *B. suis* (hogs). *Brucella abortus* causes abortion spontaneously in bovines, thus leading to major monetary losses to livestock farmers. Currently, *B. melitensis* REV.1 or *B. abortus* RB51 strains are being utilized to vaccinate caprine and ovine or bovine, respectively (Atluri et al. 2011; Shahzad et al. 2018; Dadar et al. 2019; Celli 2019). Causative agent of Brucellosis can survive for two to four months under natural environment but would die in 10-20 minutes at 60°C, by disinfectants of peroxides, iodine

or chorine. Bacteria of Brucellosis form intracellular phagocytotic vesicles to escape from the effects of antibiotics (Ugalde et al. 2000). The membranes are made of cellulose, peripheral cytoplasm membrane and the outer membrane, with outside enveloped cytoderm and peptidoglycan; it has well known antigen involved phosphatide, lipopolysaccharide and proteins distributed at out membrane. For example, different peptides have been reported, such as 10 ku/kd, 16.5 ku/kd, 19 ku/kd, 25-34 ku/kd, 31-38 ku/kd and 89 ku/kd, especially genes of omp25 and omp31 ecode 25-34 ku/kd proteins (Fig. 1).

Lipopolysaccharide of Brucella strains are in both smooth and rough forms (Corbel 1990). The rough strains, comprising no or low O polysaccharide (OPS), usually are less potent than smooth strains and are also less challenging to complement strike (Ko and Splitter 2003). However, sometimes spontaneously virulent strains, like *B. canis* and *B. ovis,* are rough stains. Virulence factor can be identified by two aspects of Brucella LPS. First, less immunogenic LPS amount in the Brucella than enterobacterial LPS. Whereas, non-pyrogenic Brucella LPS are unable to stimulate the alternate perfect route to a substantial level and is a very mild mitogen B cells (Sangari and Aguero 1996). Furthermore, 10 times more Brucella LPS is needed for interferon (IFN) production and lethality compared to bacterial endotoxins (Keleti et al. 1974; Ko and Splitter 2003). Thus, Brucella LPS low biological activity is necessary for the survival of Brucella in phagocytic cells (McQuiston et al. 1999). Second, OPS-deficient Brucella mutations are vulnerable to complement-mediated lysis and polymyxin B, as *in vivo* and *in vitro B. abortus* phosphomannomutase (pmm) transposon mutants were attenuated (Allen et al. 1998; Ko and Splitter 2003) and were susceptible to complement-mediated killing.

Brucella infection begins via ingestion or inhalation of the causative organisms through the oral, nasal, and pharyngeal cavities (Morgan and Corbel 1990). Following their entry into the mucosal epithelium, the bacteria are carried out to the regional lymph nodes, either in free form or within phagocytic cells. The propagation and proliferation of Brucella in liver, spleen, lymph nodes, mammary glands, bone marrow, and sex organs takes place through macrophages (Godfroid et al. 1998). In general, humans get *B. abortus*, *B. melitensis*, and *B. suis* infections and usual pathological manifestations include endocarditis, arthritis, spondylitis, meningitis etc. (Ko and Splitter 2003).

Pathophysiology

Brucella enters into the animal body via oral cavity; it comes-across several hurdles, like saliva that is rich source of antibodies, neutrophils, plasma cells, complement molecules, etc. After passing through the mucosal barriers of digestive system, the pathogen is defensed by the intestinal mucosa, containing proteins as well as immune cells (Mowat and Agace 2014) involved T lymphocyte and B lymphocyte in gut associated lymphoid tissue (GALT), such as mesenteric lymph nodes (MLN) and Peyer's patches (Forchielli and Walker 2005). Mucosal cells along with phagocytic cells in these tissues recognize Brucella pathogens. Dendritic cells (phagocytic cells) and macrophages (antigen-presenting cells, APCs) are capable to engulf Brucella and take them to the nearby local lymph node (López-Santiago et al. 2019). As soon as these cells engulf Brucella, APCs move to the lymph node to introduce the bacteria to the lymphocytes and then deliver it to the proper activation signal.

Host cell response to antigen

Innate immune responses

Brucella spp. infect phagocytizing cells and disrupt intracellular trafficking pathways. It allows antigen to invade defensive processes to induce an intracellular environment which is favourable for existence and multiplication of the antigen and to provide a means for propagation. After breaking the mucosal obstacles, Brucella affects intraepithelial phagocytic or submucosal cells and sabotage intracellular operating pathways (Pappas et al. 2005; Gomez et al. 2013). This pathway permits Brucella spp. to invade defensive mechanisms of host phagocytosis to create an intracellular environment that could play a role for the survival and duplication of pathogen and to support distribution of host cells (Adams 2002). The most important virulence factor of Brucella spp. is its capability of existence and multiplication within the phagocytic cells, in addition to the processes which lead to death of cells at intracellular level. Brucella spp. can affect various cells, such as epithelial cells, monocytes, macrophages, B lymphocytes, DC, etc. The antigen is depicted by macrophages, where it is recognized as intracellular processes, i.e., phagosome-lysosome fusion (Pizarro-Cerda et al. 1998) and respiratory burst via components, such as LPS and those of the type-IV secretion system (Franco et al. 2007). The Brucella spp. are intercellular bacteria, which favors their survival and tenacity by dogging the host immune system (Skendros et al. 2011; Gomez et al. 2013).

Adaptive immune responses

Adaptive immune reactions are essential for aiding the memory purposes in vaccination. In Brucellosis, purposes of the adaptive immune response can be classified into three mechanisms: i) γδ T, CD4+ and CD8+ produce IFN-γ by triggering macrophages against the bactericidal activities to obstruct the survival of the Brucella intracellularly; ii) cytotoxicity of γδ T and CD8+ cells kill the macrophages infected with the Brucella (Bessoles et al. 2011; Zheng et al. 2018); iii) Th1-type antibody isotypes, such as IgG2a and IgG3, opsonize the pathogen to enable phagocytosis (Ghaderinia and Shapouri 2017). The key role of T cells in Brucella immunity is the excretion of IFN-γ for the stimulation of cytotoxic T-lymphocyte activity and bactericidal activity in the macrophages. The importance of CD4+T and/or CD8+T cells in Brucella immunity has been presented as histocompatibility complex (MHC) class I and II (Fig. 2).

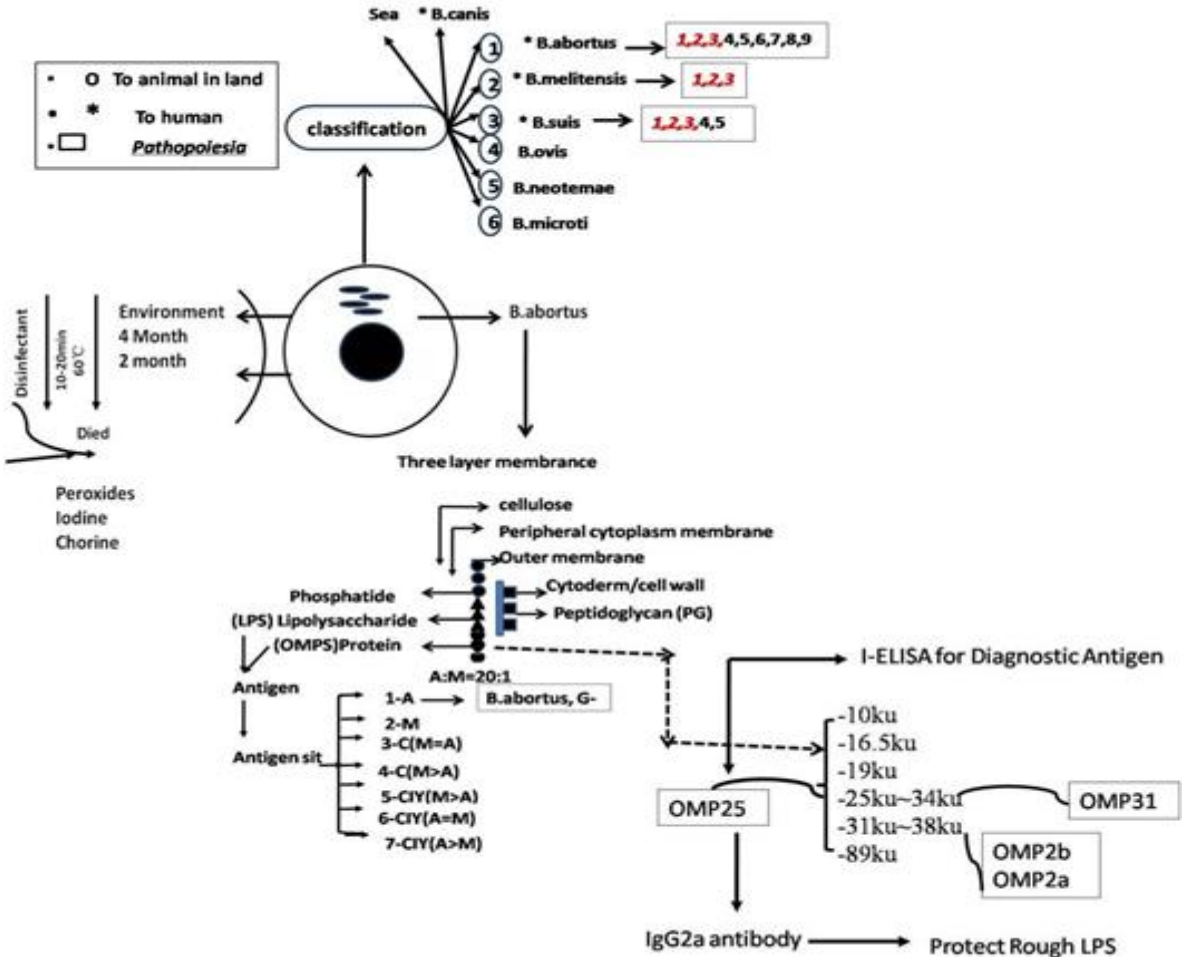

Figure 1: Pathogenesis of Brucellosis in the body.

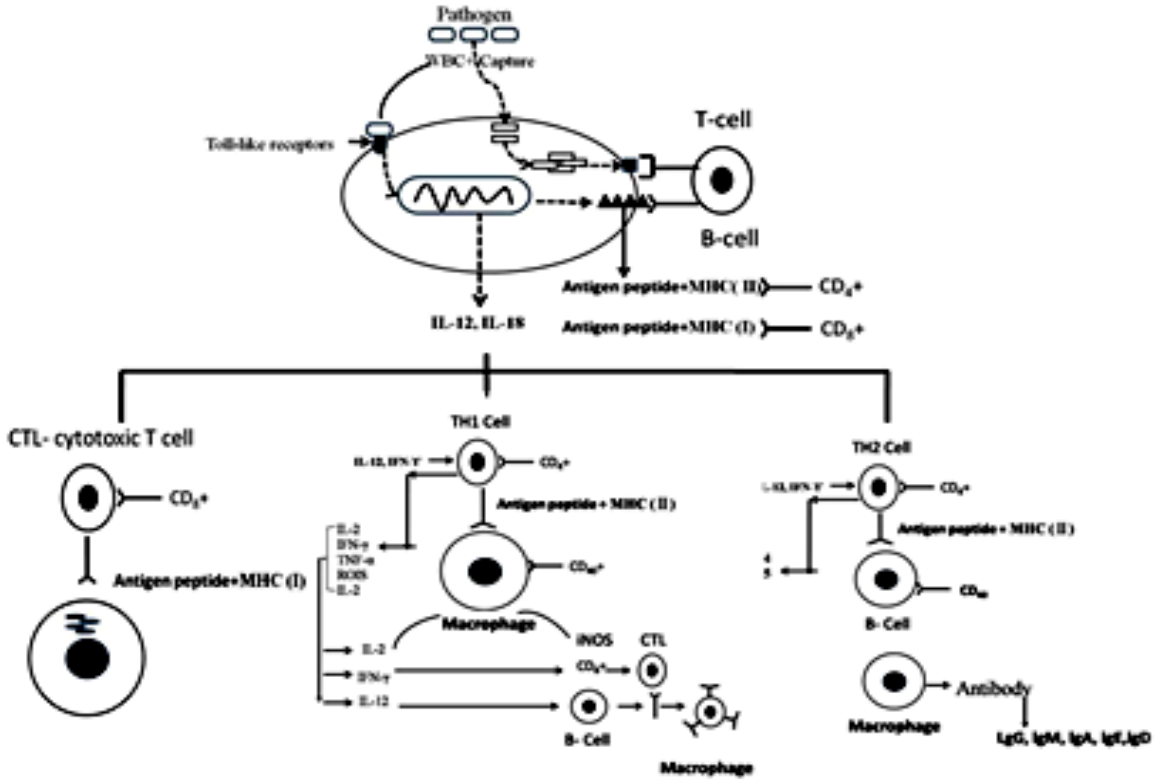

Figure 2: Mechanism of presented histocompatibility complexes.

Macrophages and T-cells play a vital role in the defense. The helper T-cell-arbitrated defense is mainly linked with a Th1 T-cell reaction and perseverance with a Th2 response (Yingst and Hoover 2003; Perkins et al. 2010; Skendros et al. 2011; Gomez et al. 2013). Precisely, results have indicated defensive aids for TNF-α, IFN-γ, and IL-12 against Brucellosis (Murphy et al. 2001; Brandao et al. 2012). Cytotoxicity of T cells and T-cell derived cytokine-mediated orchestration of the immune response in defense against the Brucellosis is important (Araya et al. 1989; Huy et al. 2021). Role of dendritic cells in adaptive and innate immunity and their survival at the level of mucosal surfaces renders them important in the study of the Brucellosis (Iwasaki 2007). The dendritic cells have been shown to be permeable to brucellae multiplication and infection (Bosio and Dow 2005). Brucella has been proved to control the reaction of these cells, i.e., dendritic cells (Iwasaki 2007; Imtiaz et al. 2018). Lastly, natural killer cells are cells with cytotoxic abilities and have ability to produce IFN, but a title role for these cells in the control of acute Brucellosis is not clear (Vivier et al. 2011; Gomez et al. 2013).

Humoral immunity

Accurate defensive processes of humoral immunity against intracellular pathogens, like Brucella, lacking in β-cell activity specify that this cell type is not essential for the defense at the level of primary infection, yet antibodies from the vaccinated and immunized or exposed animals provide necessary defense to the animals not exposed to the disease (Goenka et al. 2011). Moreover, results of a previous study indicate that antibodies possess a protecting role compared to re-infection with Brucella spp. (Gomez et al. 2013). The results further indicate that the innate immunity mechanisms, that herald expansion of humoral immunity, are adequate to overcome the primary Brucella infection and the synergetic and/or repressive impacts of antibodies need to be studied (Titball 2008).

Diagnosis

Clinically, the disease in animals often characterized by clinical signs, such as abortion, retained placenta, arthritis, orchitis, and epididymitis with excretion of the Brucella spp. in discharges and milk (Shahzad et al. 2017). There are different methods for diagnosis of Brucellosis, but the gold standard test remains the culture isolation (Ko and Splitter 2003; Gul et al. 2015). Serum agglutination tests and milk ring test are being used for the screening of the patient. Important isolation sources are milk and vaginal discharge from infected animals. Moreover, when there is abortion, then organs of aborted fetus, including stomach content, lymph nodes, etc. are the best sources for isolation of the bacteria (Singh et al. 2014). Phage typing, a very handy tool for species and biovars characterization along with biochemical tests, has been in use (Singh et al. 2014). Additionally, different serological tests involving lgM isotype, lgG1 and lg A have also been reported (Weynants et al. 1996).

With advancements in the field of diagnosis, many laboratory tests, such as 16s rRNA, ELISA and PCR across the world are in use; these tests help in the development of molecular markers which are specific and sensitive assays for the detection of Brucella spp. (Shahzad et al. 2017; Imtiaz et al. 2018). PCR–based methods that point out the molecular markers are more helpful and practical in nature than other assays and may take sometime to be fully functional and applicable in the field. PCR–based methods are quick, simple, possess high sensitivity and less hazardous (Singh et al. 2013) for Brucella detection, especially those using the 16S rRNA as targets (Shahzad et al. 2018) and the bcsp31 genes (Singh et al. 2014; Imtiaz et al. 2018) are highly sensitive for genus Brucella.

Therapy

Brucellosis is usually treated with antibiotics, like rifampin, streptomycin, gentamicin and doxycycline. However, the effect of treatment is usually limited. So, vaccine development is the best way for treatment, prevention and control of Brucellosis.

In human Brucellosis, most commonly implicated agents are *B. abortus*, *B. melitensis*, and *B. suis* (Franco et al., 2007; Wattam et al. 2009; Gomez et al. 2013). The virulence of these organisms is variable, with *B. melitensis* being at the top. Vaccination is the most effective and low-cost solution for the prevention of the disease (Oyewumi et al. 2010; Imtiaz et al. 2018). There are two main procedures to produce immune-protection against Brucellosis, vaccination of the animals/humans with live-attenuated organisms or subunit antigens (Gomez et al. 2013). However, the success of this type of immunization approach is influenced by multiple factors, including pathogen biology, efficacy, safety, and adequate levels of immunization.

The first vaccine used in cattle to control Brucellosis was the S19 vaccine (Imtiaz et al. 2018). This vaccine is a live attenuated when administered via action of cytotoxic-T-lymphocytes it produced protective immunity (Levitz and Golenbock 2012), however, it is very difficult to differentiate between infected and vaccinated animals, as both types of animals show a similar serological response (Al-Dahouk et al. 2005). Another vaccine, RB51, was unstable (Moriyon et al. 2004). Presently, live attenuated *Brucella* vaccines are being used to control the disease in animals, however, major difficulty of their wide application is about human's safety against them (Ficht et al. 2009; Goodwin Pascual 2016; Zhang et al. 2017; Lalsiamthara and Lee 2017).

Brucella melitensis Rev.1 is also a live attenuated vaccine, commonly used in animals for the control of *Brucellosis* (Levitz and Golenbock 2012; Avila-Calderon et al. 2013). The presence of smooth LPS in the vaccinal strain Rev-1 might make it difficult to differentiate between infected and vaccinated individuals, and may also interfere in the test-and-slaughter policy (Khan et al. 2017). Disadvantages of live attenuated vaccines are that, being

pathogen for humans and animals, they may i) lead to the development of resistant to streptomycin, ii) could cause abortion in pregnant animals, and iii) produce specific antibodies against LPS that may impede diagnosis (Gwida et al. 2010; Khan et al. 2017; Imtiaz et al. 2018).

It has been documented that subunit vaccines are safe and efficient against *B. abortus* in both humans and animals (Dorneles et al. 2015). Various subunit (Ghasemi et al. 2015), DNA (Leclercq et al. 2003; Al-Mariri et al. 2010) or live vector vaccines have been produced (Cabrera et al. 2009). Humoral, as well as cellular, immunity both play a significant role in protective immunity against *Brucella* infection, though cell-mediated immunity is likely to perform an important role in the safety, as *Brucella* is a pathogen that is present intracellularly (Gul et al. 2015). The IFN-γ is secrete by the CD4+ and CD8+ T lymphocytes, and is reported to play an important role in the control of Brucellosis (He et al. 2002). When DNA vaccine is used to immunize animals, both humoral, as well as cellular, immunity is produced against many pathogens (Villinger et al. 2004; Donnelly et al. 2005), thus, the effectiveness of DNA vaccine against *B. abortus* is augmented by encoding various genes, like SOD, L7/L12, and BCSP31 (Da-Hai et al. 2007; Imtiaz et al. 2018). Similarly, recombinant flagellar proteins (FlgJ and FliN) and DNA vaccine encoding BAB1_0270, BAB1_0278, BAB1_0278a (Sislema-Egas et al. 2012; Li et al. 2016) were used to produce a good immune retort and safety against *B. abortus* infectivity (Escalona et al. 2017).

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