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Review Paper

VIRULENCE FACTORS, INTRACELLULAR SURVIVABILITY AND MECHANISM OF EVASION FROM HOST IMMUNE RESPONSE BY *BRUCELLA*: AN OVERVIEW

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ABSTRACT

Brucellosis is an important zoonotic and contagious bacterial disease caused by *Brucella* spp. It is one of the most ancient diseases having a worldwide prevalence with significant human morbidity. It also causes devastating economic losses in livestock of developing countries due to reproductive failure (abortion and stillborn) and is a major hurdle in international trade. *Brucella* has the capability to induce chronic infection in animals due to lack of selective preventive measures and inefficient antimicrobial therapies, and difficulty in elimination. The virulence factors of *Brucella* are involved in intracellular survival and replication within mononuclear phagocytic cells, preferentially macrophages in the host. Furthermore, *Brucella* pathogen has developed a battery of mechanisms to evade and/or modulate both innate and adaptive immune responses in their host. Also, the stealthy nature of *Brucella* can make it able to evade from pattern recognition receptors of the innate immune arm, which can lead to its escape from intracellular destruction and to eventually replicate within phagocytic cells. All these comprehension of *Brucella* can inhabit inside the phagocytes of infected host to promote their survival, persistence and multiplication. Therefore, the analysis of the interaction between pathogen and immune response is relatively new area of intense research. In this review, we discussed the pathogenesis of *Brucella* spp. mechanisms that permit intracellular survival, subvert autophagy process and evasion to host immune responses.

Key words: Adaptive immunity**,** Autophagy, *Brucella* spp, Innate immunity, Intracellular survival, Mechanism of evasion from host, Virulence factors.

INTRODUCTION

Brucellosis is a zoonotic, contagious bacterial disease of livestock, caused by facultative intracellular Gram-negative, non-spore forming, coccobacillus of the genus *Brucella* which live in a microaerophilic environment inside its host. Presently, 10 *Brucella* species have been identified (Xavier *et al*., 2010), out of them terrestrial animals are affected by eight species which include *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis*, *B. canis*, *B. microti*, *B. neotomae* and *B. inopinata* (Scholz *et al*., 2008). Marine mammals are affected by 2 species which include *B. ceti* and *B. pinnipedialis* (Foster *et al*., 2007). Therefore, brucellosis is registered under the World Animal Health Information Database (WAHID) as a multi-species infection, infestation and disease (OIE, 2013).

These pathogens are affecting all domestic animals (cow, goat, sheep, pig and dogs), human and wild animals act as natural reservoirs (Pappas *et al*., 2005). The bacteria is transmitted to susceptible animals by consumption of contaminated food, inhalation of aerosols, direct contact with infected animals and indirect contact with infected materials which includes aborted fetuses, vaginal discharges and placental membranes or fluids (WHO, 2006). Brucellosis is prevalent globally, except in some countries, where bovine brucellosis (*B. abortus*) has been eradicated. These eradicated countries are defined by the absence of any infected animal for minimum five years. Those countries are Denmark, Australia, Finland, Cyprus, United Kingdom, Sweden, Norway, Netherlands, New Zealand and Canada (OIE, 2009). Still, brucellosis is reported in most of countries that includes Mediterranean countries of Europe, Central and South America, Mexico, Northern and Eastern Africa, Central Asia and India (Robinson, 2003).

Brucellosis causes huge economic losses worldwide in livestock industry due to loss of animal production including decreased milk production, sterility in males, infertility and abortion in females (ILRI, 2012; Saminathan *et al.*, 2016). This disease also causes significant zoonotic burden which affects human health,

and international trade consequences especially in developing countries (McDermott *et al*., 2013), Mediterranean countries (Godfroid and Kasbohrer, 2002), Southeast (Gul and Khan, 2007) and central Asia (Pappas *et al*., 2006). The new case of human brucellosis is mainly depending on the occurrence of brucellosis in livestock. Generally, the human brucellosis is not transmitted from person to person (so far, only two cases are reported). But, the higher incidence of human brucellosis is due to direct contact with natural reservoirs (cattle, sheep, goats and pigs) (Pappas *et al*., 2005) or livestock (Baldwin and Goenka, 2006; Deepthy *et al*., 2013) or livestock products (e.g., skin, wool, manure and maintenance of farm premises) (Blasco and Molina-Flores, 2011; Rodolakis, 2014). This disease can spread to wild animals by spillover from domestic animals and wild species (Godfroid, 2002; Rhyan and Spraker, 2010). The disease can be easily transmitted between wildlife, livestock and humans (Fyumagwa *et al*., 2009). Therefore, the disease is occurring from the livestock–wildlife interface, which is the areas to be targeted by the animal health authorities (Siembieda *et al*., 2011).

B. abortus, *B. melitensis* and *B. suis* causes high morbidity rate in naïve herds and much lower in chronically infected herds. In naïve cattle *B. abortus* spread rapidly, and 30-80% of the herd may abort. In domesticated pigs, the abortion rate for *B. suis* varies widely from 0 to 80%. Approximately 30-50% of all infected ram have visible lesion in epididymis. Estimation of the abortion rate has been reported to be limited (0 to 8%) (Spickler *et al*., 2010; Saminathan *et al*., 2016). Further, there is no specific treatment for control of brucellosis in livestock; although some drugs like chlortetracycline, penicillin and streptomycin have been used with fair amount of success. The *Brucella* spp. is unable to be completely eliminated by the host cellular mechanisms (Skendros *et al*., 2011) or to be eradicated by antimicrobial drugs (Skalsky *et al*., 2008; Alavi and Alavi, 2013). *Brucella* pathogens have capability to establish a chronic or persistent infection (Spera *et al*., 2014) and have evolved with sophisticated mechanisms to evade and/or modulate the immune response of their host. On account of all the above indicated reasons, brucellosis is continuously persisting in most of the areas of the world (Moreno, 2014).

The previous reviews of *Brucella* had described only the evading mechanisms of *Brucella* spp. against the host immunity. In contrast, this review have discussed the summary of all five Brucella virulence factors which are involved in the pathogenicity and intracellular survivability of bacterium, and evading from host autophagy, innate and adaptive immune responses.

Pathogenicity of *Brucella* spp: *Brucella* spp. is generally invaded into animals via mucous membrane of the gastrointestinal tract, respiratory tract, conjunctival mucosa, abraded skin and cervix of genitalia (Brinley Morgan and Corbel, 1990; Rossetti *et al*., 2013) and is endocytosed by mucosal macrophages and dendritic cells (DCs); artificial insemination (Rankin, 1965); and by inhalation (Ko and Splitter, 2003). Congenital infection may also occur in calf born from *Brucella* infected dam by intrauterine route (Ray *et al*., 1988; Radostits *et al*., 2007). This pathogen has good predilection for pregnant gravid uterus, mammary glands, testes, lymph nodes and joints (Greenfield *et al*., 2002). In the gravid uterus, a four-carbon sugar alcohol called as erythritol is produced by the fetus that serves as the preferred carbon and energy source for stimulating the growth of *B. abortus* (Meyer, 1967; Sperry and Robertson, 1975). This erythritol is playing crucial role in the localization of Brucella pathogens to these sites and successive addition of large amounts of secondary bacteria, which causes abortion. Alexander *et al.* (1981) reported that spontaneous abortions occur as a hallmark of animal brucellosis, which is due to the quantity of Brucella "endotoxin" formed in the placenta, where the bacteria reproduce to levels as high as 10^{13} bacteria/gram of tissue. Virulence character of *B. abortus* is dependent on utilization of erythritol substance initially by *B. abortus* (Williams *et al*., 1964). This erythritol is playing pivotal role in stimulation of two major virulence pathways (type IV secretion system *Vir*B and flagellar proteins) following exposure to erythritol, which suggest role of erythritol in virulence response (Petersen *et al*., 2013). In intestinal tract, these pathogens are entering through mucous surfaces: M cells in the intestine, which is a portal of entry for *Brucella* spp. (Paixao *et al*., 2009). Following entry of the bacteria, it is transported by either free or inside of phagocytic cells to the regional lymph nodes (Carvalho Neta *et al*., 2010). Once *Brucella* spp. has invaded, they have capability of surviving, and replicating in the bone marrow, liver, lymph nodes, spleen, sex organs and mammary glands (Ko and Splitter, 2003). The persistence of infection intracellularly, whether inside of phagocytic or non-phagocytic host cells, that includes macrophage and dendritic cells (DCs), as well as placental trophoblasts (Salcedo *et al*., 2008; Archambaud *et al*., 2010; Copin *et al*., 2012). Also, this pathogen can penetrate and replicate in epithelial cells and fibroblasts (Moreno, 2014).

Intracellular Trafficking: Following the *Brucella* being engulfed by macrophages or invading into non-phagocytic cells, it occupies inside a membrane-bound compartment called as *Brucella*-containing vacuole (BCV) and then convert into a replicative compartment or brucellosome (Kohler *et al*., 2002). This organism has capability to impede with intracellular trafficking by preventing fusion of the BCV with lysosome markers, in order to evade degradation within phagolysosomes by rapid acidification of the phagosome after uptake.

Therefore, the endosomal BCVs (eBCVs) are not capable to kill the bacteria due to limited fusion with late endosomal/lysosomal compartments (Starr *et al*., 2008). Also, the BCVs can resist to adverse environments and displays metabolic versatility (Atluri *et al*., 2011; Martirosyan *et al*., 2011). Some BCVs are conserving internalized *Brucella* freight from endocytic compartments and are binding with rough endoplasmic reticulum (RER), where act as niche for intracellular replication of *Brucella* (Pizarro-Cerda *et al*., 2000), constituting a distinctive aspect of the intracellular Brucella lifestyle (Fig. 1). This process can lead to change in the structural and functional features of the ER (Smith *et al.,* 2013; Salcedo *et al*., 2013a). Furthermore, this ER protect *Brucella* against not only from host humoral immunity, besides it also preserve bacteria away from host phagocytic mechanism. However, the ER is not an end stage of this intracellular journey of *Brucella* spp. In a recent study, Starr *et al*. (2012) documented that autophagy like vacuoles contribute to multiplication of permissive compartment for *Brucella* after that ER stage. Because the "*Brucella*-replicating organelle" acquires autophagic characters, it is known as autophagic BCV (aBCV). The formation of the aBCV is the final stage of intracellular lifecycle of *Brucella* and also for spreading in-between cell-to-cells (Starr *et al*., 2012).

Virulence factors as key role for stealthy to intracellular survive of *Brucella:* *Brucella* spp. is frequently called as ''nasty bugs'' based on their unusual virulence characters (Letesson *et al*., 2002). Also, none of the previous studies have explained clearly regarding the pathogenesis of *Brucella*, the molecules which are responsible to virulence properties of organism. For a long time, it was thought that *Brucella* bacterium does not contain any classical virulence factors that exist in other bacteria (Moreno and Moriyon, 2002; Fugier *et al*., 2007).

In recent several studies, it has been reported that *Brucella* is having mainly five virulence factors that are necessary for intracellular survival and infection, including *vir*B T4SS (Comerci *et al*., 2001; de Jong *et al*., 2013), cyclic *β*-glucan (Martirosyan *et al*., 2012), two-component sensory and regulatory system BvrS/BvrR (Martín-Martín *et al*., 2012), *Brucella* LPS (*Br*LPS) (Lapaque *et al*., 2005) and pathogen-associated molecular patterns (PAMPs). Additionally, some other virulence factors have been identified in *Brucella* spp, that are imperative for infection, counting BacA (Martín-Martín *et al*., 2012), BmaC (Posadas *et al*., 2012), outer membrane proteins (Omps) (Lim *et al*., 2012; Vizcaíno and Cloeckaert, 2012), SagA (Del Giudice *et al*., 2013), MucR (Mirabella *et al*., 2013), BtaE (Ruiz-Ranwez *et al*., 2013), and BetB (Lee *et al*., 2014). We have discussed first five virulence factors in this review.

(I) ***vir*B type IV secretion system (*vir*B T4SS):** In *Brucella*, T4SS is one of the main virulent factors and is encoded by the *vir*B operon which contains totally 12 genes (*Vir*B1–12) placed on chromosome II (de Jong and Tsolis, 2012). Once *B. abortus* is phagocytized by neutrophils or macrophages, T4SS is induced by *virB* proteins (Foulongne *et al*., 2000; Comerci *et al*., 2001) and phagosomal acidification, which can lead to the translocation of effector proteins into host cytosol (Boschiroli *et al*., 2002). Till date, 15 effector proteins have been identified in *Brucella* that regulates the intracellular and stealthy lifestyle of the pathogen (Salcedo *et al*., 2013; Dohmer *et al*., 2014). This process is necessary for bacteria to subvert lysosome fusion and to produce *Brucella*-containing vacuole, an organelle that allow replication and binding with the endoplasmic reticulum (Celli *et al*., 2003; Marchesini *et al*., 2011). The *vir*B operon is essential for non-opsonized *Brucella* that continues to live within the phagolysosome and to create a successful intracellular replicative compartment (Lopez-Goni and Moriyon, 2004) and modulates Brucella intracellular trafficking (Comerci *et al*., 2001; Delrue *et al*., 2001). Therefore, the T4SS plays a significant role for preventing host innate immune response and in stealthy intracellular survival during infection.

(II) **Two-component sensory and regulatory system BvrS/BvrR:** The two-component sensory and regulatory system BvrS/BvrR are significant for *Brucella* virulence, co-ordinate the outer membrane (OM) architecture, which are likelihood attitude to pathogen metabolism (Guzman-Verri *et al*., 2002; Salcedo *et al*., 2008). Out of these two components, BvrS is a sensor protein member of the histidine-kinase superfamily and BvrR is a regulator protein. This system modulates outer membrane proteins (Omp) expression which is involved in invasion of host cells (López-Goñi *et al*., 2002). Any dysfunction of the BvrR/BvrS sensory-regulatory system results in easy susceptibility of *Brucella* to bactericidal cationic peptides and complement, and increased permeability to surfactants (Sola-Landa *et al*., 1998). It changes in the bacterial outer membrane which alters cellular uptake of the organism (Manterola *et al*., 2007). Moreover, this virulence factor plays a significant role in intracellular survival of *Brucella* spp.

(III) ***Brucella* lipopolysaccharides (LPS) play imperative role to evade host immune response:** *Brucella* LPS is having two forms, which are smooth and rough strains (Corbel, 1990). Basically, rough strains accommodating less or no O polysaccharide (OPS) are less pathogenic than smooth strains and can be easily controlled by complement system (Ko and Splitter, 2003). However, *B. ovis* and *B. canis* are having naturally rough virulent strains. *Br*LPS possess an extremely resistant phenotype to cationic bactericidal peptides and create *Brucella* a lack of activator of the complement

system (Rasool *et al*., 1992; Freer *et al*., 1996). Gram negative bacteria *Brucella* LPS is made of lipid A and core oligosaccharide, which have less number of negatively charged sugars. All these features of *Br*LPS induce ability of not attach to complement, bactenecins, cathelicidins, microbicidal defensins, or any alternative cationic bactericidal molecules (Lapaque *et al*., 2005). The *Br*LPS have been described as virulence factor and plays pivotal role in *Brucella* replication and survival (Martinez *et al*., 1995). The in-built characteristics of the *Brucella* membrane envelope structure are having properties for resisting to humoral and cellular bactericidal activities of the host immune system (Martinez de Tejada *et al*., 1995; Freer *et al*., 1996). Furthermore, this *Br*LPS is having high hostility to macrophage degradation and preservation against immune responses (Forestier *et al*., 2000). The *Br*LPS changes the LPS pathogen-associated molecular pattern (PAMP) and decrease the endotoxin-related properties which are typical of LPS. In contrast to enterobacterial LPS, *Brucella* LPS is many times less active and toxic compared to *E. coli* LPS (Moriyon, 2003).

The *Br*LPS acts as a virulence factor in two aspects. Firstly, *Brucella* maintains less immunogenic LPS than enterobacterial LPS (Zahringer *et al*., 2004). So, it does not induce host immunity to prevent *Brucella* replication. Non-pyrogenic type of *Br*LPS does not induce the alternative complement pathway to any notable level and is a very fragile mitogen to murine B cells (Sangari and Aguero, 1996). Secondly, *Br*LPS induces less biological activity which might be one of the reasons for maintaining durability of these pathogens within phagocytic cells. The *Br*LPS contains a non-canonical lipid A and produce weak response to TLR-4 (Lapaque *et al*., 2006), which are contributing to *Brucella* to attain a stealthy nature at the initial stage of infection (Sengupta *et al*., 2010). The *Br*LPS stimulates less classic TLR-4 dependent activation or require no role of TLR-2 (Barquero-Calvo *et al*., 2009). This mechanism causes very restricted potential to stimulate pro-inflammatory responses in DCs (Surendran *et al*., 2012).

The LPS O chain prevents cellular apoptosis and control immune response activation (Pei *et al*., 2006). Also, the *Br*LPS are showing secondary anti-inflammatory feature, which can lead to reduced deposition of complement component C3 (Barquero-Calvo *et al*., 2007). Majority of Gram-negative bacteria are enveloped by outer membrane molecules which possess the PAMP, that are identified by innate immunity. However, that PAMP are not present on OM lipopolysaccharide, lipoproteins and flagellin of *Brucella* spp (Palacios-Chaves *et al*., 2011). So, these bacteria avoid early recognition through innate immunity.

Heat-killed smooth LPS *Brucella* strains can still limit attachment with lysosomes higher than rough mutants (Porte *et al*., 2003) that indicates an important role of the O-chain in this mechanism. Despite, the smooth LPS-dependent interruption in lysosome integration is temporary and not enough to protect *Brucella* long-term durability (Martín-Martín *et al*., 2012); it means some other bacterial factors are also necessary for finalizing the *Brucella* intracellular cycle.

(IV) **Pathogen-associated molecular patterns (PAMPs):** The PAMPs have been defined as virulence factors and that are feeble stimulators of toll-like receptors (TLRs) (Lapaque *et al*., 2009) which are contributing to *Brucella* in a stealthy nature at the initial stage of infection (Sengupta *et al*., 2010). *Brucella* spp are hidden to early detection by innate immunity, the absence of PAMP expression in the cell envelop *Brucella* OM lipopolysaccharide, ornithine-containing lipids, lipoproteins and flagellin (Lapaque *et al*., 2009), which minimally activate the innate immunity (Martirosyan *et al*., 2011). The TLRs have an extracellular leucine-rich repeat (LRR) domain which recognizes and binds to specific antigen ligands (Akira and Takeda, 2004). *Brucella* toll-interleukin receptor (TIR) domain is established in both the cytoplasmic regions of TLRs and adaptor proteins. TIR domain consist of BtpA (also known as Btp1/TcpB) and BtpB proteins, which are considered as virulence factors and are accountable for mediating the signaling cascades of innate immune recognition (Salcedo *et al*., 2013).

(V) Cyclic *β* (1–2) glucan: The cyclic *β* (1–2) glucan is having an osmoregulated periplasmic polysaccharide property and is produced through cyclic *β* (1–2) glucan synthetase enzymes, which are encoded by cgs in *Brucella* (Briones *et al*., 2001). But, it is not osmotically regulated (Briones *et al*., 1997). The cgs gene mutant *Brucella* spp is lacking cyclic *β* (1–2) glucans synthetase enzyme; that can lead to deficient in production of cyclic *β* (1–2) glucans substance. This substance is essential for inhibiting the maturation of the bacterial vacuole via preventing cholesterol-rich lipid rafts which are characterized by their enrichment in flotillin-1 and consequently prevent lysosome fusion (Dermine *et al*., 2001). The Cyclic *β* (1–2) glucan factor is responsible for the pathogen to attain its final replicating slot within endoplasmic reticulum (Arellano-Reynoso *et al*., 2005). However, this factor is not important for the trafficking of *Brucella* to the RER (Arellano-Reynoso *et al*., 2005).

These all the five virulence factors together, may contribute key virulence mechanisms for intracellular survival and multiplication of *Brucella.* Additionally, Roop *et al*. (2009) documented that some molecules of *Brucella* such as transporter-like protein BacA, flagellum-like structure and phosphatidylcholine are necessary for survival of *Brucella* inside the host cells. Furthermore, Spera *et al*. (2014) reported that chronic nature of *Brucella* is due to some virulence factors, among which are the immunomodulatory proteins such as

PrpA (proline racemase protein A), involved in the establishment of the chronic nature of the infectious condition.

Autophagy: Autophagy is defined as the process of cellular degradation, capture and removal of intracellular microbes through delivery of pathogens to lysosomes for destruction, which can lead to host intracellular innate immunity (Levine *et al*., 2011). During this process, the degraded cell could reuse valuable enzyme cofactors, amino acids, nucleotides, and lipids from digested cell proteins and organelles. Autophagy is divided into three types, which are microautophagy, chaperone-mediated autophagy, and macroautophagy (Liu *et al*., 2008). Macroautophagy, most generally called as autophagy and is significant for degrading protein aggregates, causes cell lysis (Liu *et al*., 2008), and removes intracellular organisms (Ogawa and Sasakawa, 2006; McPhee and Baehrecke, 2010).

Subversion of autophagy nucleation complexes by intracellular *Brucella* bacterium: Most of the intracellular pathogenic microbes are having different durability strategies to avoid autophagy-mediated destruction mechanisms and formation of unique intracellular survival for multiplication and persistence by modulating cellular mechanisms. Starr *et al*. (2012) documented that *B. abortus* pathogen can ‘‘hitch a ride’’ with autophagy, and prevents autophagic process to transmit between cell to cell. However, cell-to-cell spread is identified as an important step in any pathogen infection (Hybiske and Stephens, 2008). Once, the bacteria is entered into host, followed by secretion of bacterial virulence proteins into host cells by translocation and *virB* type IV secretion system this can allow bacteria to be evaded from cellular machinery and formation of BCV within host cells (Marchesini *et al*., 2011).

The BCV is playing an important role in survival of bacteria and prevents autophagy-mediated destruction of intracellular bacterium *B. abortus* by trafficking of BCV into endoplasmic reticulum (ER) from the endocytic compartments (Salcedo *et al*., 2013a). After that, the *Brucella* replication takes place in the ER, which is followed by BCV changing into a compartment with autophagic features (Starr *et al*., 2012). The aBCV formation is based on requirement of autophagy-initiation proteins including ULK1, Beclin1, ATG14L and PI3-kinase activity (Itakura and Mizushima, 2010; Matsunaga *et al*., 2010). In contrary, some of the autophagy-elongation relevant proteins are LC3B, ATG4B, ATG7, ATG16L1 and ATG5 which are not essential for aBCV formation (Collins *et al*., 2009; Nishida *et al*., 2009). Therefore, aBCV plays a significant role for entire intracellular lifecycle of *Brucella*, cell-to-cell signaling and specific subversion of autophagy nucleation complexes which can escape from host immune response and develop infection (Starr *et al*., 2012).

Innate immunity: The host immunity is divided into innate and adaptive immunity. The innate immunity is also termed as non-specific immunity, which acts as first line of bastille against infectious agents like intracellular bacteria (e.g. *Brucella* spp, *Mycobacterium* spp, *Listeria* spp, etc) (Parkin and Cohen, 2001; Kubota, 2010). The first line of phagocytic cellular populations are neutrophils, monocytes/macrophages, dendritic cells, barriers (skin), secretory molecules consisting of various chemokines, cytokines, complement system and opsonins, and natural killer cells (Dranoff, 2004; Tizard, 2012). Out of these cells, macrophages and dendritic cells (DCs) are playing a significant role in innate immunity, in recognition and in the induction of strong adaptive immunity against intracellular bacteria such as *Brucella* spp (Baldwin and Goenka, 2006; Olsen *et al*., 2010).

***Brucella*: a stealthy bacterium baffle the innate immune response:** Once the pathogen invades in to vertebrate host, it is initially detected by the host innate immunity via pattern-recognition receptors (PRRs). These PRRs play crucial role in detection or recognition of minute amounts of microbial components which are known as PAMPs (Vilaysane and Muruve, 2009). These PAMPs are consisting of membrane localized TLRs (Iwasaki and Medzhitov, 2004), complement (Snyderman *et al*., 1968), cytosolic nucleotide binding and oligomerization domain-like receptors (NLRs) (Franchi *et al*., 2008), RIG-I receptors (RLRs) or NLRs identifying RNA motifs, peptidoglycan or lipopolysaccharide. All these receptors are expressed by only bacteria or viruses but not by the host (Medzhitov and Janeway, 1997).

These pathogen recognition or detection receptors plays pivotal role in the host that can easily discriminate bacteria from viral and parasitic agents through PAMPs (Aderem, 2003; Hoebe *et al*., 2004; Malik *et al*., 2013). Additionally, some toll like receptors including TLR1, TLR2, TLR4, TLR5 and TLR6 recognize bacteria (Iwasaki and Medzhitov, 2004). These PAMPs are mandatory for preventing intracellular survival of the microorganisms, which escape from host innate immune system (Janeway and Medzhitov, 2002). Even though, many microorganisms have developed various mechanisms to overcome host defense, including both innate and acquired immunity (Finlay and McFadden, 2006).

As a successful pathogen, *Brucella* has adopted a stealthy approach to manage the innate immune system, averting sustained recognition by PRRs and consequent strong inflammatory responses (Barquero-Calvo *et al*., 2007; Martirosyan *et al*., 2011). Therefore, some of the pathogens are displaying changed PAMPs to elude detection by host innate immunity (Campos *et al*., 2014).

Additionally, *Brucella* inhibits phagolysosome fusion apoptosis, and downregulates antigen presentation (Skendros *et al*., 2011), which can lead to their escape from effector immune responses.

The best example of *Brucella* with altered PAMPs is LPS of *Brucella*, which allows bacteria to hide from the innate immune system (Pappas *et al*., 2005). Billard *et al*. (2007) and Salcedo *et al*. (2008) documented that *Brucella* spp is escaping from strong recognition by the innate immune system by preventing full activation of infected macrophages and to maintain infected DCs in an intermediate maturation stage. These bacteria will bind with RER, preserving them from components of the humoral immunity and also protecting them away from the intracellular endocytic bactericidal mechanisms. *Brucella* spp is conserved in intracellular environment which protects it from host humoral immunity, phagocytic mechanism and thus avoids destruction resulting in evasion of innate immunity (Lapaque *et al*., 2005). Therefore, based on stealthy nature of *Brucella* which evades immune detection by host, it is referred as ‘‘Mr Hides’’ (Gorvel, 2008).

A**daptive immunity:** The second barricade in the host arm is the adaptive immunity, which is otherwise known as antigen-specific immune response or specific immunity. It consist of T lymphocytes, which are responsible for cytokine production and cytotoxicity (cellular immunity), and B lymphocytes that are responsible for antibody production (humoral immunity) (Parkin and Cohen, 2001). The adaptive immunity usually develops within five or six days following the presentation of antigen epitopes via APCs and innate immunity. Besides, this response is specific to pathogens and the remarkable property of “memory”. On the contrary, the innate system is triggered within minutes to hours after pathogens entry to host and targets pathogens non-specifically.

Evasion from adaptive immune response by intracellular *Brucella* bacterium: Adaptive immunity usually starts after activation of innate immunity. The adaptive immune response helps to remove the infection and creates memory component to that specific antigen in the host, which is an essential property in long lasting vaccination response. Generally, *Brucella* inhabits within the macrophages, and interferon-γ (IFN-γ) stimulates macrophages to perform potent brucellicidal functions (Sathiyaseelan *et al*., 2000). Macrophage-derived cytokines which are interleukin 1 (IL-1), IL-12, and tumor necrosis factor alpha (TNF-α) plays important role in control of early *Brucella* spp. infection by IFN-γ pathway (Zhan and Cheers, 1994). IFN-γ secreted by $CD8^+$ T cells or the B cell-mediated humoral immunity play important role for clearance of brucellosis (Vitry *et al*., 2012).

The adaptive immune response can control *Brucella* infection by three methods. Firstly, IFN-γ activates the bactericidal function on *Brucella* residing in the macrophages in order to prevent the intracellular survival and IFN-γ is produced by $CD4^+$, $CD8^+$, and γδ T cells. Secondly, cytotoxicity mechanism of $CD8^+$ and γδ T cells destroys *Brucella* infected macrophages. Thirdly, Th1-mediated antibody isotypes, such as IgG2a and IgG3 engulf the pathogen to promote phagocytosis and degradative endocytic compartments. Moreover, cytokines (IL-12, IFN-γ and TNF-α) are plays pivotal roles to elicit immune responses to *Brucella* infection (Billard *et al*., 2007; Salcedo *et al*., 2008). Furthermore, the adaptive immune responses are also controlled by DCs which are called as mediators of pathogen recognition. These DCs are mostly situated at the site of bacterial entry; also, and possess the capability to move from peripheral tissues to secondary lymphoid organs to evoke primary T cell responses and stimulate immunity (Kapsenberg, 2003). The pathogen causes morphological and biochemical changes on infected DCs, such as cytokines IL-12, TNF-α secretion and increased expression of surface co-stimulatory and MHC class II molecules (Watowich and Liu, 2010; Schmid *et al*., 2010). This activation of DCs, called as maturation process, play a significant role for efficient T-cell priming and pathogen elimination (Joffre *et al*., 2009).

Inevitably, few of the successful pathogens like *Mycobacterium tuberculosis* has found specific ways to corrupt DC function (Khan *et al*., 2011; Prendergast and Kirman, 2013). The *Brucella* was observed in actively multiplying DCs both *in vitro* (Salcedo *et al*., 2008) and *in vivo* (Archambaud *et al*., 2010). Despite, the *Brucella* infected DCs showed low expression of MHC class II, CD80 and CD86 co-stimulatory molecules. It can lead to inhibition of DCs maturation (Heller *et al*., 2012), characterized by the absence of secretion of cytokines such as TNF-α, IL-12 and incompetent antigen presentation to naive T cells (Billard *et al*., 2007; Salcedo *et al*., 2008).

In immunosuppressed animals, lack of clearance of bacteria from infected spleen, liver and lymph nodes (Hort *et al*., 2003) is observed due to the defective cellular activation of $CD8^+$ T lymphocytes and gradual reduction of macrophages and DC maturation (Rolan and Tsolis, 2007). Furthermore, *Brucella* prevents the development of a defensive Th1 immune response by hindering the secretion of IL-12 and by inhibiting the T-cell stimulatory activity of infected DCs (Salcedo *et al*., 2008). The DCs full activation is controlled by *Brucella* and then this microbe uses the tolerogenic properties of DCs to overthrow immune responses. Therefore, *Brucella*-infected DCs exhibit an intermediate level of maturation, which leads to tolerance stimulation in tissues and to development of a long lasting chronic infection has *Brucella* infected human monocytes/macrophages

which inhibited the expression of MHC-II molecules and antigen presentation to $CD4^+$ T lymphocytes (Barrionuevo *et al*., 2008). Also, the *Br*LPS changes MHC-II presentation pathway by formation of LPS macrodomains at APCs plasma membrane (Forestier *et al*., 2000). All this strategies results survival and multiplication of *B. abortus* within the infected macrophages for long duration by indirectly changing the $CD4^+$ T cell function.

It is not well known regarding the mechanism that *B. abortus* is able to directly interfere with T lymphocytes. Some of previous results showed that *B. abortus*-infected individuals had decreased Th1 response during chronic phase of the infection Giambartolomei *et al*. (2002). Skendros *et al*. (2008) also reported similar observation that patients with chronic brucellosis have diminished percentage of peripheral $CD4^+$ and $CD25^+$ T lymphocytes. Therefore, *B. abortus* could be minimizing the T lymphocyte responses through decreasing the number of T lymphocytes in chronic infection. Additionally, several microorganisms may cause apoptosis of host defense cells like T lymphocytes and it can lead to persistent infection in the host (Gao and Abu Kwaik, 2000). For example, Vela´squez *et al*. (2012) reported that *B. abortus* causes apoptosis of human T lymphocytes. So, this pathogen can directly interact with T lymphocytes thus modulating their life cycle. Moreover, this pathogen also evades T lymphocyte response both directly and indirectly for surviving intracellularly. Further advancement is going in the molecular characterization of virulence factors of *B. abortus* (PrpA) that stimulates a transient anergic state of the immune system (Spera *et al*., 2006) and B-cell proliferation (Spera *et al*., 2013) which are promoting the establishment of the chronic phase.

Brucella infection causes increase in intracellular calcium level, which plays as an essential virulence factor for the intracellular survival of *Brucella* in macrophages (Cui *et al*., 2014). *Brucella* infected macrophages have expressed zinc-finger protein A20 which are necessary for *Brucella* intracellular growth through preventing macrophage activation and apoptosis in the TNF receptor1 signaling pathway (Wei *et al*., 2015). Therefore, *Brucella* has developed mechanisms to hinder with information transmission from the innate to the adaptive immune system in order to avoid host immune response.

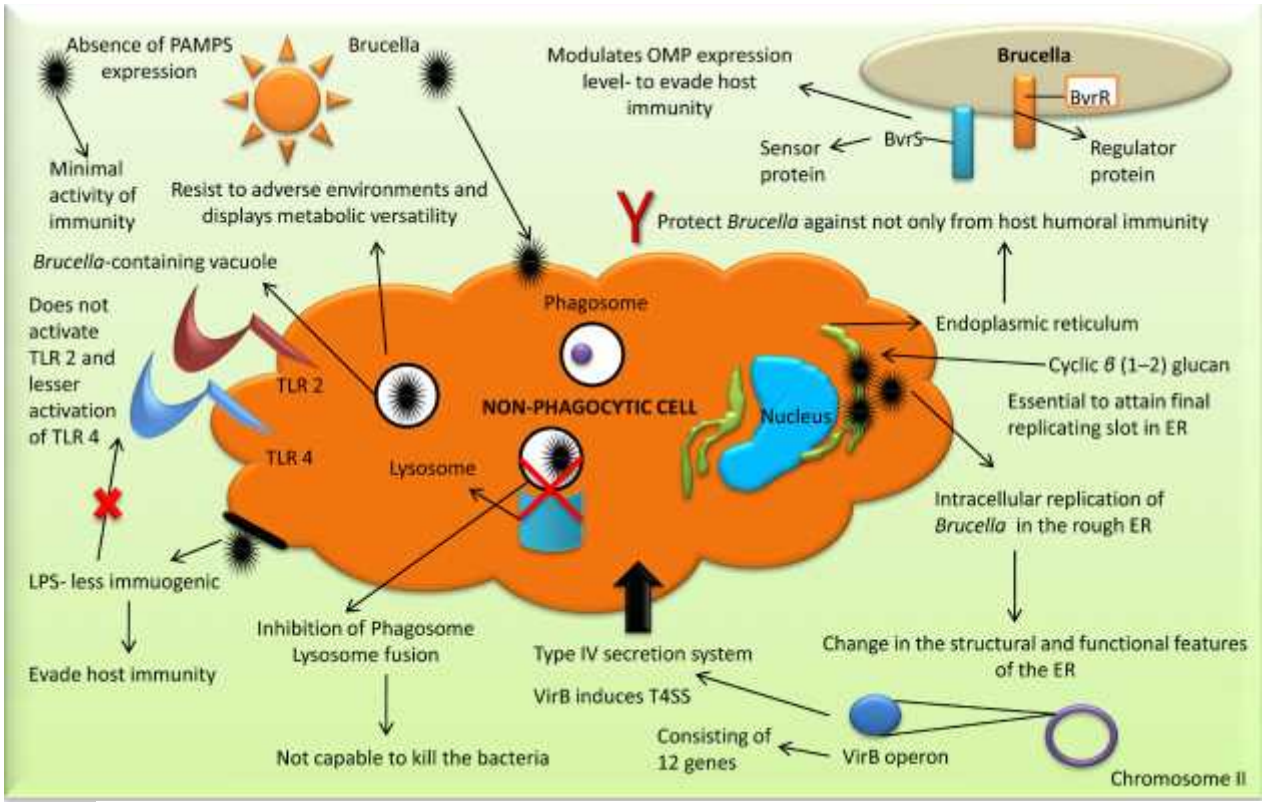

Figure 1. An overview of virulence factors, intracellular survivability and mechanism of evasion of Brucella from host immune response: *Brucella* are engulfed by macrophage, once internalized, Brucella are trafficked through a vesicle sharing markers with an early endosome. Later the *Brucella* is found in a compartment resembling a late endosome, which forms endosomal BCVs (eBCVs) that resist to lysosomal or adverse environmental condition. Some eBCVs are able to bind with endoplasmic reticulum, which helps for BCV

replication process. Virulence factors like *vir*B type IV secretion system, two-component sensory and regulatory system BvrS/BvrR, *Brucella* lipopolysaccharides, PAMPs and Cyclic β (1–2) glucan play a major role in pathogenesis.

Conclusion: Brucellosis is one of the important zoonotic problems for human health and causes major economic loss in animal husbandry sector. Most of the disease causative pathogens are efficiently removed by host immune cells, but *Brucella* has capability to establish a chronic or persistent infection. Five virulence factors of *Brucella* are involved mainly for its intracellular survivability and replication within mononuclear phagocyte cells, which hampers the intracellular trafficking and ability to prevent recognition by the host defense arm. The *Brucella* pathogen has developed a battery of mechanisms to evade and/or modulate both innate and adaptive immune response in their host. Further studies in this interesting area would help in designing suitable prevention and control measures to counter this important zoonotic pathogen.

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