# Pathogenesis of *Brucella* spp.

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**Abstract:** Brucellosis is one of the most important zoonotic diseases worldwide, resulting in serious economic losses and public health issues. It is caused by intracellular Gram-negative bacteria of the genus *Brucella*, which are responsible for a debilitating disease in humans and a chronic infection in domestic animals. The present article considers the pathogenesis of *Brucella* spp., with the goal to cover clinical aspects of the disease in the different mammalian species along with the target cells used by this pathogen to survive inside the host. Additionally, important molecular mechanisms used by *Brucella* to invade and persist inside the hosts target cells are also discussed.

**Keywords:** Brucellosis, pathogenesis, *Brucella*.

## INTRODUCTION

Brucellosis is one of the most widespread zoonotic diseases globally, with an estimated 500,000 new human cases each year. The disease is caused by Gram negative bacteria of the genus *Brucella*, which are facultative intracellular coccobacilli that belong to the α2-Proteobacteriacea family [1]. In spite of more than 94% similarity amongst the members of the genus [2,3], bacteria of the genus *Brucella* have different host preferences. Therefore, *Brucella* spp. are capable of causing disease in a variety of animal species, including humans (Table **1**).

**Table 1. Zoonotic Potential and Host Preference of *Brucella* Species**

| Species | Zoonotic Potential | Host Preference |
|---|---|---|
| *Brucella melitensis* | High | Sheep, goat |
| *Brucella abortus* | Moderate | Cattle |
| *Brucella suis* | Moderate | Pig |
| *Brucella canis* | Mild | Dog |
| *Brucella ovis* | Absent | Sheep |
| *Brucella neotomae* | Absent | Desert wood rat (*Neotomae lepida)* |
| *Brucella ceti* | Mild | Cetaceans |
| *Brucella pinnipedialis* | Mild | Seals |
| *Brucella microti* | Absent | Common voles (*Microtus arvalis*) |

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Division of the genus into six classical species *Brucella*, namely *B. melitensis, B. abortus, B. suis, B. canis, B. ovis* and *B. neotomae*, is still widely used due to historical and clinical reasons [4]. *B. melitensis, B. suis* and *B. abortus* are considered the most pathogenic species for humans and have small ruminants, pigs and cattle as preferential hosts, respectively [5]. In addition, two recently identified *Brucella* species isolated from marine mammals, *B. ceti* and *B. pinnipedialis*, can also cause human brucellosis [6]. Importantly, *B. canis*, a pathogen of dogs, has a comparatively low zoonotic potential, while *B. neotomae* and *B. ovis*, that infect desert rats and sheep and, respectively, are not associated with human disease [5].

Human brucellosis is considered as a life-threatening debilitating disease characterized by weakness, fever, malaise, arthritis, osteomyelitis, endocarditis or meningoencephalitis [7]. In domestic animals, the disease occurs as a chronic infection that results in placentitis and abortion in pregnant females [8,9] or orchitis and epididymitis in males [9].

An important aspect of *Brucella* infection is its ability to persist and replicate within phagocytic cells of the reticuloendothelial system as well as in non-phagocytic cells such as trophoblasts. This ability involves a temporary fusion of the *Brucella*-containing vacuole with the lysosome, and subsequent exclusion of the lysosomal proteins [10]. Following this process, the *Brucella*-containing vacuole becomes associated with the endoplasmic reticulum. These endoplasmic reticulum-associated compartments are the niche for intracellular replication of *Brucella* in macrophages, epithelial cell lines and placental trophoblasts [11-14]. Once inside this compartment, the bacteria can establish chronic infection.

This review describes the pathogenesis of *Brucella* infection in the light of recently generated knowledge regarding host-pathogen interactions and molecular mechanisms of intracellular survival and pathogenesis.

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## HOST-PATHOGEN INTERACTIONS AND DISEASE MANIFESTATION

Human brucellosis is considered one of the most important zoonotic diseases worldwide [15, 16]. Although *B. abortus, B. suis* and *B. canis* are potential agents of this disease, *B. melitensis* is considered the most virulent *Brucella* for humans with a few organisms (10 to 100) being sufficient to cause a debilitating chronic infection [17]. In most cases, human infections occur through ingestion of contaminated milk and unpasteurized dairy products. However, occupational exposure of mucosa or skin abrasions to fluids and tissues from aborted fetuses of infected animals or carcass is also an important source of infection [17, 18]. The efficient transmission of *Brucella via* inhalation of contaminated dust or aerosols makes brucellosis one of the most common laboratory-acquired infections worldwide and spurred the original weaponization of *Brucella* spp. by the United States and the former Soviet Union in the 1950's.

Human brucellosis is a life-threatening disease that may have variable clinical presentations [19]. After exposure to the bacteria, clinical manifestations may appear within 5 to 60 days [20]. Most infected patients present with acute disease consisting of general symptoms, such as fever, malaise, sweats and lymphadenopathy and/or hepatosplenomegaly. However, a subset of patients develops chronic brucellosis, a more severe form of the disease that can be associated with osteo-articular signs including spondylitis, arthritis and osteomyelitis, or genitourinary changes, such as orchitis, epididymitis, glomerulonephritis and kidney abscesses [18, 19]. Life-threatening complications comprise, in descending order of frequency, neurobrucellosis, liver abscesses, and endocarditis [17].

Brucellosis in small ruminants is mainly caused by *B. melitensis,* although this pathogen may also infect cattle and other ruminants [21]. This pathogen, which has three different biovars [22], is endemic in several parts of the world, particularly biovar 3 in Mediterranean and Middle Eastern countries [23]. Some Latin American countries are also seriously affected by biovar 1, especially Mexico, Peru and Northern Argentina [24]. *B. melitensis* infection of goats and sheep is characterized by abortion, reduced milk yield, and orchitis. Although sexually mature animals of both genders are equally susceptible to the disease, the predominant sign of acute infection is reproductive failure with abortion in the last trimester and birth of weak offspring. In small ruminants, transmission of the disease often occurs by contact of susceptible animals with contaminated secretions from the female genital tract [25]. Approximately two thirds of acute natural *B. melitensis* infections of goats during pregnancy lead to infection of the udder and excretion of the bacteria in milk during the subsequent lactation. Progressively, intermittent shedding of the agent in milk occurs in animals with persistent infection of the udder. *B. melitensis* may cause inflammation of the mammary tissue, which is the most probable cause of reduced milk production in infected animals. However, clinical signs of mastitis are usually not detectable in naturally infected goats [25].

Cattle are considered to be the preferential host for *B. abortus*, but the organism can also affect buffaloes, camels, deer, dogs, horses, goats, sheep, and man [26]. This *Brucella* species is classified into seven different biovars, namely biovars 1-6 and 9. Outbreaks of brucellosis in dairy herds result in decreased milk production, while they increase somatic cell count in milk, abortion and post-partum metritis [27]. Therefore, bovine brucellosis is primarily a disease of cows, with the organism being isolated from the udder, uterus, and lymphoid organs of infected animals [8, 28]. Although abortion during the last trimester of gestation is considered the predominant clinical sign of the disease, infected cows usually abort only once, giving birth to weak or even healthy calves in subsequent gestations. Importantly, some infected cows will not exhibit any symptoms of the disease and give birth to normal calves [29]. *B. abortus*-induced abortion is associated with necro-hemorrhagic placentitis and fetal lesions, particularly fibrinous pleuritis and pericarditis, and interstitial pneumonia [28]. As described for *B. melitensis*, *B. abortus* infection may lead to a mild to moderate interstitial mastitis, resulting in intermittent shedding of the pathogen in the milk [28]. Transmission of bovine brucellosis occurs mainly after abortion or parturition of infected cows when susceptible cattle may have contact with contaminated fetuses, fetal membranes, and uterine secretions [29]. In bulls, *B. abortus* is a common cause of orchitis that is often associated with a seminal vesiculitis and epididymitis. Infected bulls usually do not play a major role in spreading the disease. Infection in males may result in either temporary or permanent infertility, depending on the intensity of the lesions [30].

Porcine brucellosis caused by *B. suis* biovars 1, 2 and 3, is considered an important reemerging disease of domestic and wild pigs. However, this pathogen may also affect other species such as cattle, horses, rabbits, dogs, and humans [31, 32]. Biovars 1 and 3, which have pathogenic potential for humans, occur in Europe, North, South and Central America, Southern Asia and Pacific islands [33]. *B. suis* infection in pigs often does not result in clinical signs, making clinical diagnosis a very difficult task. In clinical cases, porcine brucellosis is primarily characterized by a genital disease with abortions. However, the bacteria may also affect other organs, particularly bones and joints [34]. Therefore, the disease is considered a herd problem with pigs of all ages being affected, albeit with a higher incidence of infection in adults. Transmission of the disease in pigs occurs by both venereal and oral routes, with *B. suis* being secreted in large numbers, for long periods in the semen and urine as well as in uterine discharges, and milk [25]. The most important clinical signs in sows are infertility, irregular estrus, abortion in any stage of gestation, and birth of weak piglets with a high neonatal mortality rate. Porcine brucellosis is the only disease in which reproductive failure in sows is accompanied by orchitis in boars and osteo-articular disorders such as arthritis, osteomyelitis, spondylitis, and paralysis [35]. Albeit orchitis and epididimitis are the most common lesions in boars, the infection occasionally may be restricted to sexual glands and may not result in impaired fertility. However, even these asymptomatic cases may result in shedding of the organism in the semen with potential for venereal transmission of infection [36].

Canine brucellosis, caused by *B. canis,* usually affects domestic dogs, wild carnivores, and rarely other domestic animals [37]. The disease occurs mostly in Central and South America. *B. canis* is also considered a zoonotic agent, but

infections are uncommon and usually mild. Most natural human infections have been acquired through close contact with infected dogs or accidental laboratory contamination [37]. In dogs, transmission of the disease usually occurs by breeding or ingestion of contaminated placental tissues, aborted fetuses or vaginal secretions from infected bitches. Importantly, *B. canis* may be shed for long periods in semen or vaginal secretion after occurrence of abortion [38, 39]. In bitches, the predominant clinical sign is abortion after 45-55 days of gestation. Occasionally, early embryonic death and reabsorption, or abortion 10-20 days after mating, may occur [37]. Infected males have unilateral or bilateral epididymitis and orchitis as the most important clinical signs, often leading to infertility. Semen from infected dogs usually contains large numbers of abnormal sperm and inflammatory cells, especially during the first three months after infection. Chronically infected males may have azospermia, or reduced numbers of immature sperm [40]. In contrast to other *Brucella* infections, *B. canis* infection of dogs results in prolonged bacteremia. Therefore, blood culture is a valuable diagnostic approach in canine brucellosis, as opposed to other *Brucella*/host combinations [38].

Ovine brucellosis caused by *B. ovis* infection is a disease of sheep that occurs in most sheep-raising areas worldwide such as Australia, New Zealand, North and South America, South Africa, and many countries in Europe [41]. *B. ovis* primarily causes epididymitis in sexually mature rams, and occasionally abortion in ewes [42]. Poor semen quality characterized by decreased sperm concentration and sperm abnormalities is often associated with early infections (Fig. **1**) [43]. With the progression of the disease, palpable lesions may develop in the epididymis, which may be unilaterally or, occasionally, bilaterally affected [42]. It is noteworthy that chronically affected rams usually have regression of the lesions in the epidydimis and most infected rams never develop any clinical sign of the disease [41]. In addition, those asymptomatic rams may shed *B. ovis* in the semen for long periods, thus increasing the risk of spreading infection in the herd [41]. Transmission can occur by direct contact between rams kept in the same premises for prolonged periods of time or through ewes that have mated with an infected ram prior to a susceptible one during the same mating season [44, 45]. In ewes, *B. ovis* can uncommonly cause abortion associated with placentitis beginning at 30 days of gestation. Infected ewes may give birth to weak lambs with a high neonatal mortality rate [46].

Since the 1990's, marine strains of *Brucella* have been isolated from a variety of marine mammal species, including seal (*Phoca vitulina*), dolphins (*Tursiops truncatus*; *Delphinus delphis*; *Lagenorhynchus acutus*; *Stenella coeruleoalba*), whale (*Balaenoptera acutorostrata*), and other species [47-51]. First classified as *B. maris*, the marine isolates of *Brucella* are now putatively divided into two species: *B. ceti* and *B. pinnipedialis*, referring to isolates from cetaceans and seals, respectively [52]. As described for other species of the genus, some marine isolates are capable of infecting humans, with reported cases associated with neurological disorders [53, 54]. It is believed that transmission to human occurs through direct contact with marine mammals or ingestion of infected animals. However, there are reports of human brucellosis caused by marine isolates in which there was no evidence of contact of the patient with marine animals [53-55], which raises the question of whether additional reservoirs of these *Brucella* species contribute to human infections.

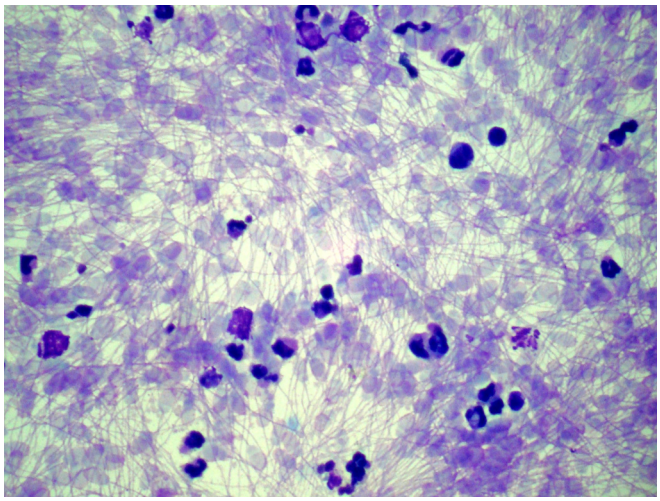

**Fig. (1).** Semen smear from a ram infected with *Brucella ovis* containing several inflammatory cells (predominantly neutrophils).

In marine mammals infected with *Brucella*, transmission of the disease may occur through mucosa and injured skin, direct contact, or by the oral route due to ingestion of other infected marine mammals [56]. Vertical or horizontal transmission to fetus also has to be considered as a route of infection, since *Brucella* has been isolated in fetal tissues and in milk from dolphins [54, 57]. Marine animals affected by brucellosis develop pathological changes that include skin abscesses, hepatic and splenic necrosis or/and histiocytic inflammation, meningitis, discospondylitis, and abortion [49]. Importantly, non suppurative meningoencephalitis has been described as the most consistent histological change in dolphins with neurological signs and positive serology and immumohistochemistry to *Brucella* sp. [54, 58]. Additionally, marine *Brucella* species are capable of infecting terrestrial mammal species as demonstrated by experimental infection of cattle [59].

Recently, a previously undescribed *Brucella* species was associated, with two cases of second semester of gestation stillbirth in a baboon colony [60]. Apparently less pathogenic species of *Brucella* have also been reported. *B. neotomae,* firstly discovered in 1947 [61], is known to infect only the desert wood rat under natural conditions in the USA, and no other cases in addition to the original isolation have been reported. In 2000, a new *Brucella* isolate named *B. microti* was isolated from systemically infected common voles (*Microtus arvalis*) in South Moravia, Czech Republic [62]. Later on, *B. microti* was isolated from mandibular lymph nodes of wild red foxes (*Vulpes vulpes*) hunted in Austria [63]. Those facts indicate that there is still a wide range of information to be explored regarding the genus *Brucella* and its host range. Further, the pathogenic potential of these recent isolates for humans or other (domestic?) animal species have yet to be investigated.

## TARGET CELLS

*Brucella* spp. is capable of invading and surviving in both phagocytic [64] and non-phagocytic host cells [65].

Macrophages (Fig. **2A**), dendritic cells (DCs), and trophoblasts (Fig. **2B**) represent the major target cells for *Brucella*, according to clinical manifestation of brucellosis in experimental and natural hosts, characterized by persistent infectious in lymphoid tissues and inflammatory lesions in the reproductive tract of pregnant females [28, 66, 67]. Bacterial entrance, survival and replication have been intensively investigated in phagocytes, but these mechanisms are poorly characterized in trophoblasts, which represents an important gap in our understanding of the disease and transmission in the natural host species.

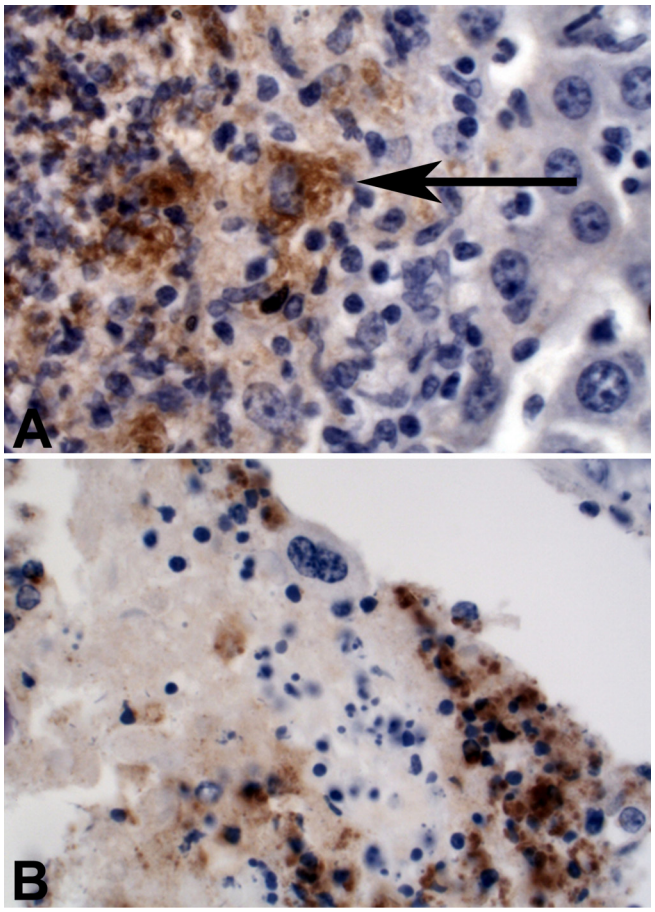


**Fig. (2).** (**A**) Mouse. Hepatic microgranuloma with immunostained *Brucella* spp. (arrow). (**B**) Cow. Chorionic membrane with trophoblastic cells containing myriad of predominantly intracellular immunostained *Brucella abortus*. 50x.

In order to reach its target cells, *Brucella* needs to cross mucosal barriers of the respiratory, genitourinary or digestive tract, where it undergoes phagocytosis by resident macrophages and DCs, resulting in dissemination of the organism to lymphoid and reproductive organs [68, 69]. Invasion through the digestive tract is associated with epithelial transmigration of bacteria preferentially through M cells. Intra-epithelial phagocytes may also transport *Brucella* from the intestinal lumen to the lamina propria [69-71].

In macrophages, *Brucella* is internalized by phagocytosis that requires a moderate recruitment of actin filaments upon activation during interaction of *Brucella* and receptors on the surface of the macrophage cell membrane. Opsonized *Brucella* is internalized *via* FC or complement receptors whereas non-opsonized bacteria interact with lectin and fibronectin receptors [72]. Non-opsonized *Brucella* can survive and replicate inside cells; in contrast, opsonization of bacteria or IFNγ-activation of macrophages enhance intracellular killing of bacteria inside the host cell [73, 74]. Lipids rafts, which are cholesterol-rich microdomains in the cell membrane of macrophages, also participate in bacterial internalization, and additionally these rafts contribute to directing intracellular trafficking of bacteria [75, 76]. Soon after internalization, the *Brucella*-containing phagosome interacts with early and late endosomes. The majority of phagocytosed *Brucella* is destroyed by bactericidal action of free radicals of oxygen, nitric oxide and enzymes inside phagolysosomes. However, a certain number of bacteria resists these bactericidal mechanisms, and after transient fusion with the lysosome can actively exclude lysosomal proteins and redirect the BCV to the endoplasmic reticulum, where the organism is capable of replicating [10, 14, 77-80]. Importantly, acidification of the *Brucella*-containing phagosome does not injure the bacteria, but it triggers expression of bacterial genes that are essential for intracellular survival during the early stages of infection [81, 82].

DCs are other phagocytes for which *Brucella* has a marked tropism, being infected more efficiently than macrophages. Bacteria are able to survive and replicate in DCs similarly to macrophages, although intracellular growth tends to be more prominent in DCs [67]. Furthermore, bacteria can inhibit maturation of DCs compromising DC antigen presentation and cytokine secretion [70, 83, 84]. As a result, DCs have two important aspects which transform them in excellent carriers for *Brucella*: high tolerance for bacteria development, and migratory properties which could support pathogen spreading [67]. Importantly, DC assays have been conducted in human and mice cell lines. Therefore, differences in the pathogen behavior regarding these cells related to the host species may be observed.

The behavior of *Brucella* in non-phagocytic cells has been better investigated in epithelial cell lines [13, 85-88]. Although *Brucella* is capable of invading epithelial [85, 86, 88] and trophoblatic cells [89, 90], it is much less invasive than other facultative intracellular bacterial pathogens such as *Salmonella enterica* [91]. No specific receptors for *Brucella* have been identified in non-phagocytic cells, but there is evidence that some *Brucella* molecules can contribute to cellular adhesion and invasion [92-94]. *Brucella* invades epithelial cells *via* recruitment of actin filaments and rearrangement of the host cell membrane. Internalization of the organism involves activation of regulatory proteins of the cytoskeleton, named small GTPases of the subfamily Rho, including Rho, Rac and Cdc42. In addition, mediators of cell signaling pathways, such as cyclic GMP, PIP3-kinase, tyrosine kinase, and mitogen-activated protein (MAP) kinases, also play a role in internalization acting as second messengers for signals from the GTPases [88, 95]. Intracellular trafficking of *Brucella* in epithelial cells is similar to that observed in phagocytes [64, 65]. Fluorescence microscopy studies demonstrated that *Brucella* traffics initially in early phagosome expressing small GTP-binding protein Rab 5 and early endosomal antigen 1 (EEA1) markers [13, 95], progressing towards autophagosomes containing the lysosome associated membrane glycoprotein 1 (LAMP1) and then to the rough

endoplasmic reticulum-associated compartments that have markers such as calreticulin and sec61β [13, 65, 87, 96]. Therefore, *Brucella* traffics from the initial phagosome towards a vacuole composed by membranes of the rough endoplasmic reticulum, which is the optimal site for its replication [12, 13, 87].

Trophoblastic cells are key target cell of *Brucella* infection during late phase of gestation in ruminants [27, 28, 68]. Growth of *Brucella* inside trophoblasts is apparently enhanced in the presence of high concentrations of steroid hormones and erythritol during the final third of gestation [97, 98]. The capacity to replicate rapidly and extensively in trophoblasts can compromise the integrity of the placenta and infection of fetus, resulting in abortion or birth of weak offspring [28, 99]. In addition, hormonal changes in infected placentas may affect the occurrence of abortion since an increase in prostaglandin 2α, estrogen and cortisol, and decrease in progesterone levels mimic what happens during parturition [100].

## MOLECULAR PATHOGENESIS

The pathogenic potential of *Brucella* spp. is highly dependent on its ability to enter and survive within host cells. *Brucella* does not have classic virulence factors such as exotoxins, capsule, or endotoxic lipopolysaccharide (LPS) [101]. The major virulence mechanisms of *Brucella* already identified are those required for host cell invasion and intracellular survival or replication [81, 102-104].

A successful entry of *Brucella* into the host is a crucial step in establishing infection. Considering that the digestive tract is the main entrance route of *Brucella,* some studies investigated possible virulence factors involved on successful infection through the digestive tract [71, 105-107]. The genes that encode urease are required for establishment of infection by *B. suis*, *B. abortus* and *B. melitensis* [71, 105, 106]. Urease is a multi-subunit enzyme, involved in nitrogen metabolism, which causes increase of pH due ammonia production as a result of urea hydrolysis [108]. *Brucella* has two non-adjacent operons of urease and the *ure1* is responsible for urease activity and its inactivation cause attenuation of strains in mice when the organism is inoculated *via* the digestive tract [105, 106]. The role of *Brucella* urease in inhibition of phagosome acidification by ammonia has not been demonstrated [106]. Apparently, the type IV secretion system (T4SS) and LPS are also required to establishment of gastrointestinal infection, although mutant strains still have ability to go across intestinal mucosa *via* M cells similar to virulent strain (Fig. **3**) [71]. Bile salt hydrolases may be other bacterial enzyme essential to *Brucella* infecting mice *via* oral route. The deletion of choloylglycine hydrolase gene in *B. abortus* causes impairment of bacterial growth in medium containing bile salts and attenuation in mice after ten days of intragastric infection [107].

During internalization, *Brucella* relies on a two-component regulator system, BvrR/BvrS that regulates expression of outer membrane proteins (OMPs) involved in invasion of host cells [102, 109]. The two components of this system are BvrR, a regulator protein, and BvrS, a sensor protein with histidine-kinase activity. This regulator system is required for recruitment of GTPases and actin filaments, and for maintaining the integrity of the bacterial outer membrane [102, 109]. Mutant strains lacking BvrR/BvrS are unable to invade phagocytic and non-phagocytic cells because they do not recruit GTPases, particularly Cdc42. This regulator system is also important for intracellular survival, since mutant strains are unable to inhibit phagosome-lysosome fusion [102, 110].

An additional mechanism employed by *Brucella* to avoid fusion of the bacteria-containing vacuole with lysosomes in macrophages are cyclic β1,2-glucans [103]. Glucans are constituents of the bacterial periplasm with osmoregulatory and cholesterol sequestering activity and are required for survival of *Brucella* in non-phagocytic cells and *in vivo* in mice. Cyclic β1,2-glucans of *Brucella* prevent phagosome maturation by interfering with lipid rafts, thus altering protein expression in the vacuolar membrane and excluding lysosomal proteins from the BCV [10, 103, 111].

LPS is another virulence factor of *Brucella* that contributes to initial survival of bacteria in macrophages [104]. LPS is essential for the functional and structural integrity of the outer membrane of gram-negative bacteria. Characterization of the LPS phenotype of *Brucella* spp. as smooth or rough depends on the presence or absence of the surface-exposed O-polysaccharide chain, respectively. The O-polysaccharide plays a major role in virulence associated with smooth LPS since mutant rough strains are defective for survival in macrophage cultures, as well as *in vivo* in mice [104, 112, 113]. Although rough mutants derived from virulent smooth *Brucella* mutant strains generally invade host cells more efficiently than smooth strains, they are less able to survive within the cell. However, some rough strains may be naturally virulent [85, 86]. Intracellular replication of smooth *Brucella* strains may reflect the role of smooth LPS in the early development of the *Brucella*-containing phagosome. *Brucella* can block maturation of phagosome through the interaction of smooth LPS with lipid rafts, which contributes to inhibition of phagosome-lysosome fusion [79]. Furthermore, smooth LPS provides resistance to complement and antimicrobial peptides such as α-defensins and lactoferrins [104, 114-116]. Smooth LPS also confers resistance to nitric oxide, free radicals, and lysozyme, important antibacterial mechanisms of macrophages and neutrophils [104, 112, 117-119]. Therefore, *Brucella* smooth LPS may be considered a virulence factor required for resistance against both extra- and intracellular antimicrobial mechanisms of the host.

There is evidence that other outer membrane components may play role as virulence factors of *Brucella* spp. A mutant strain of *B. abortus* lacking Omp25 is attenuated in pregnant heifers and it is not capable of replicating in bovine phagocytes and trophoblasts [120]. Conversely, Omp25 mutant strains of *B. ovis* has an impaired ability to invade non-phagocytic cells, whereas it is capable of surviving in epithelial and macrophage cell lines [121], and it is attenuated in mice [122]. Phosphatidylcholine, a common component of the eukaryotic lipid membrane, is the major phospholipid in outer membrane of *Brucella* and other α-proteobacteria, whereas it is typically absent from most prokaryotes. Mutant strains unable to produce phosphatidylcholine have a reduced ability to avoid the

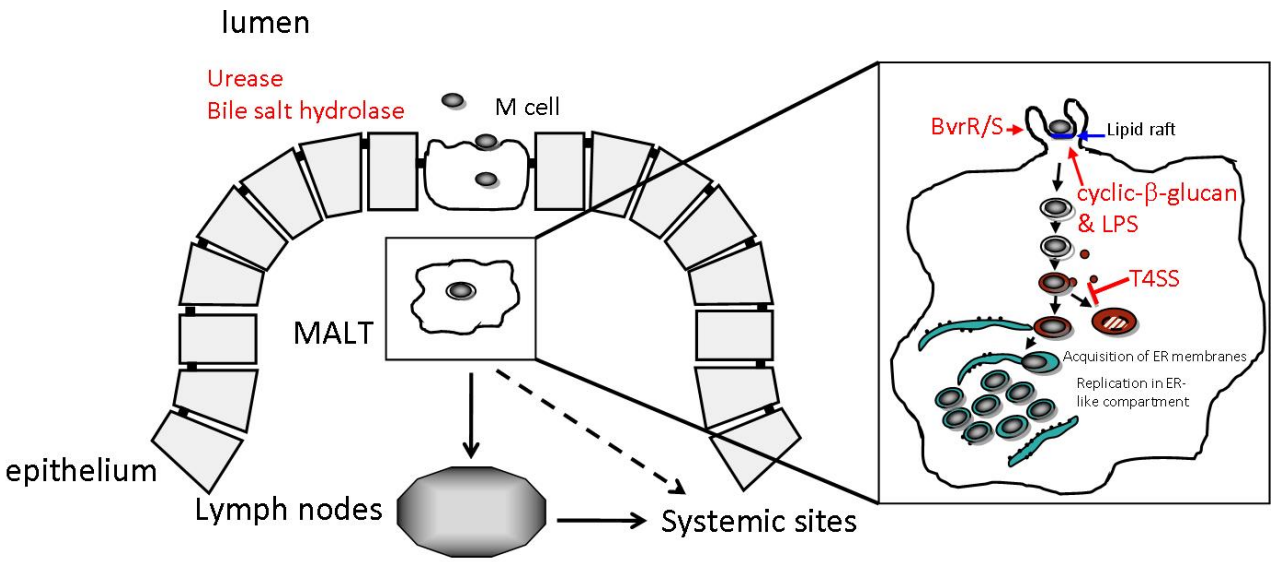


**Fig. (3).** Schematic representation of *Brucella* invasion through the digestive tract. Entry is through M cells and subsequently the bacteria are taken up by macrophages of the mucosa associated lymphoid tissue (MALT). These macrophage transport the bacteria to the lymph nodes and on to systemic sites. Blown up macrophage shows trafficking within the macrophage from entry *via* lipid rafts, through the endosomal pathway to the ER-like compartment in which *Brucella* replicates [10]. In red are *Brucella* virulence factors that are involved in establishing infection.

fusion with lysosomes in macrophages [123] and are attenuated in mice [123, 124].

The *Brucella* type IV secretion system (T4SS), encoded by the virB1-virB12 genes, is required for intracellular growth of *Brucella* in phagocytic and non-phagocytic cells [96, 125-130]. An orthologous T4SS was originally identified in the plant pathogen *Agrobacterium tumefaciens*, and it was later on recognized as virulence factor of animal pathogens such as *Legionella pneumophila* and *Bordetella pertussis*. The T4SS is a virulence factor characterized by a transporter apparatus localized in the outer membrane. It is able to translocate bacterial DNA or effector proteins into target host cells [125]. The T4SS of *Brucella* apparently does not secret DNA, and only recently some secreted molecules have been identified [131]. Effector molecules secreted by the *Brucella* T4SS possibly play a role in phagosome maturation and trafficking of the *Brucella*-containing vacuole towards its replication niche [132] since a *Brucella virB* mutant strain is not capable of reaching the endoplasmic reticulum [14]. This critical role of the *Brucella* T4SS reflects in a marked impairment of persistence of *virB* mutant *Brucella* strains *in vivo* in both mice [129, 133], and goats [134]. Mutant *Brucella* strains lacking a functional T4SS have a different protein profile when compared to wild type strains [135, 136]. Furthermore, a functional T4SS may be required for induction of expression of several *Brucella* proteins that contribute to its virulence [136], although the mechanism by which this might occur is unknown. Although the T4SS is absolutely required for intracellular survival and replication, it is apparently not required for invasion and initial intracellular survival [64]. However, there are evidences that a *virB* mutant strain of *B. abortus* is not capable of invading trophoblasts *in vivo* in pregnant mice [137].

## BRUCELLA AND INNATE IMMUNITY

Interestingly, it has been demonstrated that a functional T4SS is not only required for establishment of chronic infection in mice, but it is also essential for induction of inflammatory and immune response in splenocytes of the mouse *in vivo* [138]. In addition, a functional T4SS is required to induce B cell maturation, T CD4+ cell activation and initial secretion of IL12 and IFNγ [139, 140]. Finally, a recent study indicated that a functional T4SS is also essential for induction of microgranuloma formation in the spleen of infected mice, a typical histopathological lesion of *Brucella* infection [141]. Since *Brucella* spp. have been shown to localize to microgranulomas in infected tissue (Fig. **2A**), the T4SS-induced inflammation could trigger migration of cells involved in microgranuloma formation. These microgranulomas may represent a site for persistence of *Brucella* in the natural host reservoir until the next breeding season.

While the T4SS elicits a low proinflammatory response, *Brucella* has additional strategies both to evade, and to actively prevent inflammatory responses at the site of entrance in the host. Limiting the inflammatory response to invading bacteria is likely to attenuate or delay the onset of an effective immune response, thereby allowing bacterial invasion, survival and dissemination in the host [70, 71, 84, 89, 142]. Compared to other invasive bacteria such as *Salmonella spp., Brucella* triggers a weak inflammatory response. Two distinct mechanisms are known to contribute to evasion of innate immunity. First, *Brucella* spp. produce a lipopolysaccharide that has only weak endotoxic properties, compared to that of other bacteria, which allow it to escape detection by Toll-like receptors (TLR) of the innate immune system in sentinel cells of musosal surfaces [76]. Second, *Brucella* can actively interfere with the generation of

inflammatory responses by producing a protein that inhibits TLR-dependent cellular signaling pathways leading to inflammation. Since these same TLR signaling pathways are required to trigger maturation of DCs and proinflammatory cytokine/chemokine secretion, blocking this pathway is likely to affect both innate and adaptive immune responses to *Brucella* [70, 84]. A third immune-inhibitory activity of *Brucella* is the ability to block production of tumor necrosis factor alpha (TNF-α) [143]. Although the molecular basis for this activity is unknown, it also seems to play a role in inhibiting DC maturation [83].

## PERSPECTIVE

*Brucella* species are zoonotic pathogens causing persistent disease, both in the natural hosts, and in the incidental human host. Recent advances have shown that this pathogen elicits only moderate inflammatory responses, which is likely the result of strategies both to "hide" from immune detection and to actively suppress generation of host immune responses. Advances made using model systems in the laboratory have increased our understanding of pathogenic mechanisms that allow these organisms to survive in macrophages and dendritic cells--the very cells designed to eliminate invading bacteria and prime adaptive immunity. The molecular interactions with host cell components that lead to intracellular persistence are an exciting area of ongoing work. However, some questions about the biology of *Brucella* spp. remain to be answered, such as how the bacteria penetrate mucosal surfaces, and the molecular basis for targeting of *Brucella* spp. to tissues of the reproductive tract. A better understanding of these two processes may help us understand why the different *Brucella* species exhibit their particular host preference.

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