CHAPTER 13

BRUCELLOSIS: VIRULENCE FACTORS, PATHOGENICITY AND TREATMENT

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INTRODUCTION

There are nearly 100 different types of organisms that can cause human diseases (Balloux and van Dorp, 2017). Brucellosis is considered as one of important zoonotic diseases, especially in developing countries that is caused by Br. species such as *Br.suis*, *Br.melitensis* and *Br.abortus* are the most important members of the family because they can cause human disease (Franc et al. 2018; González-Espinoza et al. 2021). The British army surgeon David Bruce (1855-1931) isolated a coccobacillus called *Micrococcus melitensis* from some spleen tissue of a man who had died of "Malta Fever" in 1886. The disease was endemic but confused with other diseases, particularly malaria. In Malta during (1901-1906), annually reported 652 civilian cases and 605 military cases, with mortality rates of 10.4% and 2.3% respectively (Rahman et al. 2006; Liu 2015).

The disease in humans is prevalent in those who consume goat milk and have other close contacts with goats. The organism was quickly isolated from the goats. Similar microbes were isolated from cow udder in 1897, as well as from swine udder in 1914 (Ndegwa et al. 2001; Zhao et al. 2015). In approximately 1920, Brucella was renamed and each species was given its own name: *Br. melitensis, B. abortus,* and *Br. suis.* There are not all pathogens that are specific to a particular species e.g., cattle can be infected with *B. suis*. There have been numerous names for the disease, with "undulant fever" becoming dominant in the United States until the 1940s when was named Brucellosis (Alton and Forsyth 1996).

An Overview of Brucella's Characteristics

Brucella species are microorganisms that measure between 0.5-0.7 x 0.6-0.15 micrometers and are gram-negative coccobacilli. Usually, single forms are common; pairs and chains are rare. These bacteria do not produce spores, do not have capsules or flagella, and cannot move. They do not harbor plasmids naturally, even though they readily accept plasmids with broad target ranges (Alton and Forsyth 1996).

Partially acid-fast, do not decolorize when treated with 0.5% acetic acid used in modified Ziehl-Neelsen (MZN), retain carbol fuchsin, and exhibit red coloration under a microscope (al Dahouk et al. 2003; Köse et al. 2005).

Ideal temperature for growth is 37°C, with growth taking place between (20°C - 40°C), and a pH of 6.6-7.4. The majority of them are aerobes, although some species such as *Br. ovis* and *Br. abortus* need an environment with added carbon dioxide (5-10%). Brucella species are included in fastidious bacterial species that require rich culture medium to thrive (Alton and Forsyth 1996).

Growth occurs on Brucella agar, Trypticase soy agar, sheep blood agar, MacConkey agar and standard nutritional agar at (25-42 ° C). Colonies on translucid media are convex, transparent and have an entire edge. After two -three days of incubation of a fresh inoculum they are usually small (0.5–1.0 mm), but variations depend on the strain and medium (Boussetta 1991; de Miguel et al. 2011; Ledwaba et al. 2020) . Cultures can be identified as Brucella by examining colonial morphology, staining, and slides agglutination with anti-Brucella serum, smooth or rough. Many of the Brucella strains are catalase- and superoxide dismutase-positive; they are also mostly oxidase-positive. With cytochrome-based electron transport, aerobic metabolism is the mode of metabolism (Araj 2010; Tekle et al. 2019)

In conventional media, brucellae mostly use oxidative metabolism and show little activity with carbohydrates, though they can hydrolyze urea in many cases (Padilla Poester et al. 2014; Tekle et al. 2019).

There are no classical pathogenic factors produced by Brucella organisms, such as exotoxin, cytolysin, exoenzymes, exoproteins, capsules, plasmids, fimbriae, and drug-resistant forms (Głowacka et al. 2018)

Types and Classification of Antigens

It is still believed that Brucella species, despite a century of research and extensive analysis, are major animal pathogens that cause Brucellosis. These gram-negative bacteria affect various terrestrial and aquatic mammals, such as sheep, goats, cattle, dogs, swine, dolphins, whales, seals and desert woodrats. Within the Brucella genus, there are six species and these species are classified primarily based on their pathogenicity and host preferences (Cardoso et al. 2006). *Br. abortus* affects cattle, *Br.melitensis* affects sheep and goats, *Br.ovis*

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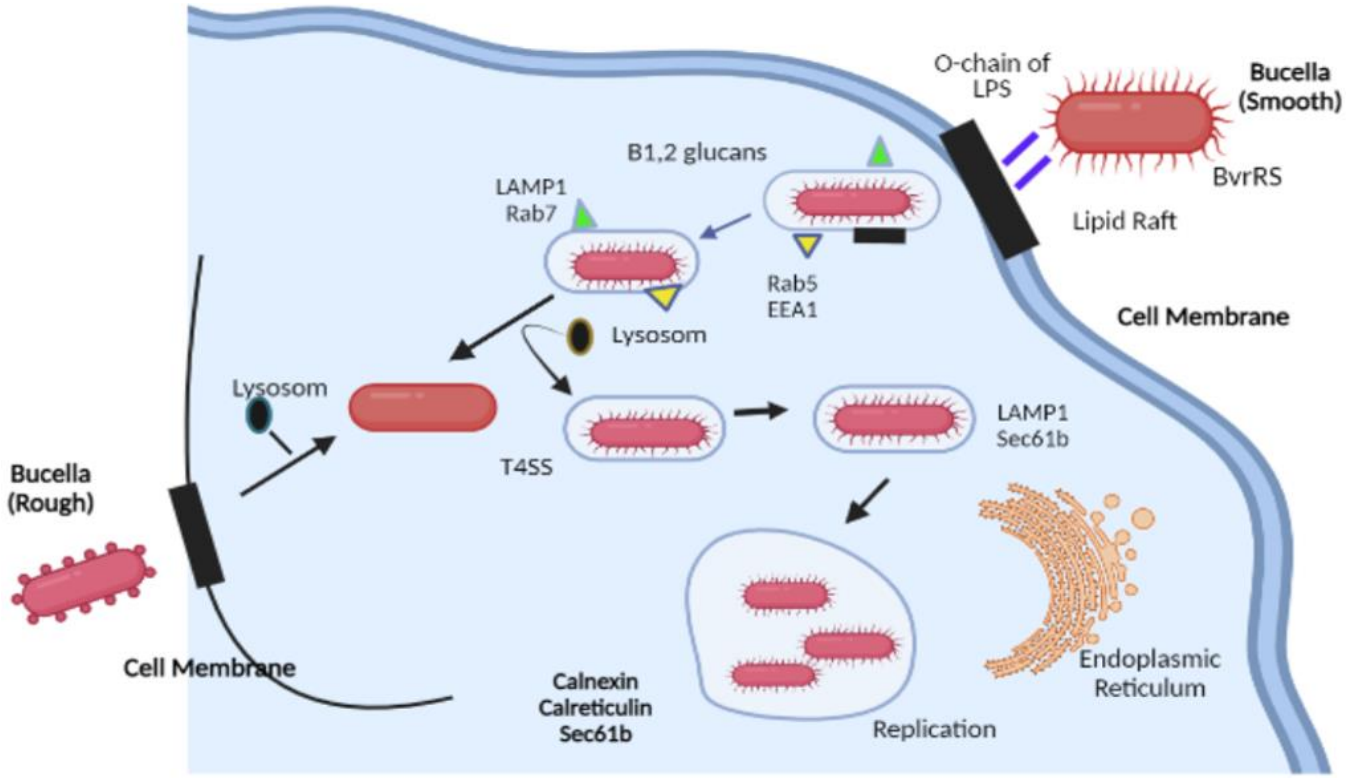

Figure 1: Invasion and Intracellular Trafficking of Mammalian Cell by Brucella (Created in BioRender.com)

Table 1: Brucella species and biovars with host range

Brucella species	Biovar	Host
Br.abortus	9	Cattle, dogs, horses, sheep and man
Br.suis	5	Pigs, cattle, dogs, hares and man
Br.melitensis	3	Sheep, goats, cattle and man
Br.ovis		Sheep
Br.canis		Dogs and man
Br.neotomae		Desert wood rat

affects sheep, *Br.suis* affects pigs, *Br canis* affects dogs, and *Br. neotomae* affects wood desert rats. Recent isolates from human *(Br.inopinata),* (*Br.inopinata*), two aquatic mammals (*Br.pinnipedialis* and *Br.ceti*), and a common vole (*Br.microti*) are now recognized as new species in the genus (de Figueiredo et al. 2015). Biovars occur in some species (Table 1) most of these species infect specific hosts.

Current research suggests that species and biovars can be differentiated based on lipopolysaccharide antigens, CO2 requirement, dye sensitivity, phage typing, hydrogen sulfide production and metabolic properties (Alton et al. 1989; Morgan 1990)

This bacterium is similar to other Gram-negative bacteria in its dominant lipopolysaccharide (LPS) component and three main protein groups in its outer cell membrane (Maldonado et al. 2016)

There are smooth and rough Brucella abortus, melitensis, and suis strains, with smooth LPS (S-LPS) and rough LPS (R-LPS) as major surface antigens. *Br.ovis* and *Br.canis* are naturally rough species that express R-LPS (Cardoso et al. 2006; Maldonado et al.2016)

The LPS of brucellae with smooth colonies has two kinds of O chains. Antigens A and M correspond to *Br. abortus* and *Br.melitensis*, respectively. (Informally,' since some *Br.abortus*, biovars carry M antigens while *Br.melitensis* carry A antigens) They are both homopolymers of 4,6-dideoxy-4-formamido-d-mannopyranose, but the A chain is linked 2-1, whereas the M chain often has three-one linkages. According to routine serology, smooth brucellae cross-react almost entirely with the same species, but not with the rough Brucella, and vice versa. Cross-absorption of A and M monoclonal sera produces monoclonal antibodies specific for each antigen, indicating that each chain contains a distinct epitope (de Figueiredo et al. 2015)

The Clinical Manifestations

Infection with Brucella causes Brucellosis, which is commonly found in domestic, wild, and feral animals, and some strains are pathogenic to humans. The Brucella genus causes the disease (Brucellosis), which is widespread and causes infertility and abortion in domestic and wild animals (Alton and Forsyth, 1996)

The manifestations of brucellosis in humans are typically variable. Sometimes it is difficult to determine how long the incubation period is, but it is usually between two and four weeks. It may occur slowly or suddenly. Subclinical infections are common, and it is characterized by undulant fever (38°C to 40°C), polyarthritis, meningitis, pneumonia, anorexia, endocarditis, splenomegaly, depression, weight loss, and hepatomegaly. There is unusually severe leg and back pain, excessive sweating, and fatigue and other less common clinical manifestations (Sauret and Vilissova 2002). A human with an untreated infection will suffer from a debilitating flu-like illness with chronic complications (González-Espinoza et al. 2021)

In domestic animals, like cattle, sheep, goats, and swine, significant effect includes abortion and metritis in females, and orchiepididymitis and infertility in males, resulting in reduced fertility and a significant decline in milk production (McDermott et al. 2013; Elderbrook et al. 2019).

Epidemiology

Brucellosis is an endemic zoonotic disease typically found in the Middle East, Central Asia, South and Central America, Africa, the Mediterranean region (Portugal, Spain, Greece), and other parts of the world with a high dairy consumption and little of animal health protection (Gwida et al. 2010; Fouskis et al. 2018). There are several species of animals that are infected with Br. abortus and Br. suis, including bears, bisons, caribous, camelids, elks, ferrets, deer, foxes, rats, and wolves, as well as dolphins, dugongs, manatees, otters, and sea porpoises (Głowacka et al. 2018)

People become infected through various routes, including contaminated dairy products, non-pasteurized cheeses, handling of infected animals, and exposure to uterine secretions or aborted fetuses at work (Khurana et al. 2021). As human brucellosis is essentially a zoonotic disease, control and prevention of brucellosis in animals is essential for eradicating the disease in man (Gwida et al. 2010).

Studies have documented *Br.melitensis* infection in ibex and chamois in the Alps (Assenga et al. 2015). There has not yet been evidence of prevalence of *Br. ovis* or *Br. canis* in European animals. Br. pinnipedialis and Br. ceti appear to be the most common causes of infections in marine animals. In contrast, *Br. pinnipedialis* and *Br. ceti* appear to be the most common causes of infections in fish. Birds are not affected by brucella infection. It is spread through close contact and sharing of pastures (Makita et al. 2011; Muma et al. 2007).

Brucella is an accidental human pathogen that is spread mainly through direct contact with infected animals, inhalation of airborne agents, or consumption of contaminated dairy products (Godfroid et al. 2013; López-Santiago et al. 2019). It is possible that human-to-human transmission can happen during organ transplantation, blood transfusions, or vertical transmission through breastfeeding (Ay et al. 2016). Several Brucella species can be fatal to humans, including *Br.melitensis*, *Br.suis, Br. abortus*, and *Br. canis* (López-Santiago et al. 2019).

Virulence Factors

There are several virulence factors of Brucella species, contributed to its pathogenicity like:

Lipopolisaccharide (LPS)

Lipopolysaccharide from Brucella is unique and nonclassical, unlike Gram-negative bacteria such as Escherichia coli (Cardoso et al. 2006; von Bargen et al. 2012). Brucella LPS have distinct structures and properties, several of these properties may contribute to Brucella's ability to survive and replicate inside cells (Lapaque et al. 2005). Brucella is known for their high resistance to macrophage degradation, low endotoxicity, and resistance to immune response (Moreno et al. 1981).

Brucella lipopolysaccharide is less active and less toxic than classical Escherichia coli. In addition, classical LPS induces high pyrogenicity, while nonclassical LPS induces low pyrogenicity, which is a weak indicator of tumor necrosis factor (Christopher et al. 2010). Three features distinguish lipid A in *Br. abortus* from other Gram-negatives: diaminoglucose instead of glucosamine, more extended acyl groups, and lipid A is connected to the core by amide bonds, instead ester and amide bounds (Conde-Álvarez et al. 2012; Corbel 1997). There are three components of smooth LPS (S-LPS) found in smooth colonies: i) lipid A, which contains two types of aminoglycosides in addition to β--hydroxymiristic acid; ii) a core of mannose, glucose, and quinovosamine; and iii) 4-formamido-4,6-dideoxymannose with an O-chain (Alton and Forsyth 1996; Lapaque et al. 2005)

R-LPSs differ from S-LPSs in that their O chains are absent or reduced(Conde-Álvarez et al. 2012) . The O-chains of bacteria attach to lipid rafts on the macrophage surface and enter the cell. Brucella strains with R-LPS, such as *Br. ovis*, and *Br. canis* are not associated with lipid rafts and rapidly adhere to lysosomes. The O-chain of S-LPS strains inhibits host cell apoptosis through interaction with TNF-α (tumor necrosis factor). Therefore, dying cells do not produce specific factors. Thus, Brucellae cannot be detected by the immune system (Celli et al. 2003)

T4SS (Type IV Secretion System)

T4SS is a multiprotein complex involved in the secretion of macromolecules by bacteria. Brucella species have the virB operon, which encodes 12 proteins (11 860 bp), which has many similarities to the T4SS found in rhizobia, such as in phytopathogenic Agrobacterium (Boschiroli et al. 2002)

The expression of the virB operon is controlled by the VjbR quorum sensing regulator (Sieira et al. 2010). Brucella species that lack the VirB gene are unable to replicate within the endoplasmic reticulum, either because they are incapable of reaching the ER or because they are incapable of multiplying within (Boschiroli et al. 2002)

As part of Brucella-containing vacuoles (BCVs), Brucella rods are localized in macrophages; these organelles interact with the ER and are thought to be responsible for the formation of specific compartments. T4SS, which is virB's secretion system, is important for the acquisition of an endoplasmic reticulum membrane (Xiong et al. 2021)

The Superoxide Dismutase and Catalase Enzymes

The macrophage produces reactive oxygen intermediates (ROI) in response to Brucella consumption, which is the primary mechanism by which Brucella is destroyed, and prevents Brucella from replicating in the cell (Gee et al. 2004; Seleem et al. 2008).

Reactive oxygen intermediates are O_2– (superoxide), H_2O_2 - (hydrogen peroxide), and OH– (hydroxyl radical) which are extremely detrimental for the structure of the cell. A major defense against reactive oxygen intermediates is the production of enzymes. These enzymes include catalase and superoxide dismutase (Hasanuzzaman et al. 2020)

This enzyme is encoded by the *sod* (metalloenzyme) sequence. A variety of metals are found at the active sites of enzymes, such as iron, magnesium, zinc, and copper. As a result, SOD converts O^{2-} (superoxide) into H_2O_2 (hydrogen peroxide) and O_2 (oxygen) - transferring from one molecule to another ($2O^{2-} + 2H^{+} \rightarrow H_2O_2 + O_2$).

Water and oxygen are produced by catalase, an enzyme that breaks down hydrogen peroxide. Combined with Cu-Zn SOD, catalase activity is restricted to the periplasmic space, which leaves external sources of ROI unchanged. Other enzymes can compensate for the absence of catalase in catalase mutants, for example alkyl hydroperoxide reductase or enzymes involved in DNA repair. The sequence encoding this enzyme is similar to that of the Escherichia coli *katE* gene of Escherichia coli. (Gee

et al. 2004) found that Br. abortus, *Br. melitensis*, and *Br. suis* make more catalase when exposed to increasing amounts of H_2O_2.

Cyclic β-1-2-glucans (CβG)

Brucella CβG is an OPG (Osmoregulated Periplasmic Glucan) II family. By interacting with lipid rafts on macrophage surfaces, Brucella abortus CβG influences intracellular trafficking. Glucans are essential to the bonding of phagosomes and lysosomes. Mutants are destroyed in phagolysosomes and cannot reproduce. A further advantage of mutants treated with CβG is that they control lysosome fusion and vacuole maturation, which allows them to replicate when reach the endoplasmic reticulum (Roset et al. 2014)

Urease

There are two different urease operons in two different genomes of Brucella. The enzyme breaks down the urea into carbonic acid and ammonium, increasing the pH. This characteristic allows it to survive in acidic environments. Two urea operons (ure-1 and ure-2) are found on the 1 chromosome. The ure-1 and ure-2 genes encode structural proteins: ureA, ureB, ureC, and accessory protein genes: ureD, ureE, ureF, ureG. It has been suggested that the urease enzyme protects Brucella from destruction during its passes through the gastrointestinal tract (stomach), particularly when it enters orally (López-Santiago et al. 2019). Brucella species able to produce urea, except *Br. ovis* (al Dahouk et al. 2010).

The Cytochrome Oxidase Enzyme

Brucella can survive in macrophages in low-oxygen environments through the action of the enzyme cytochrome oxidase. In the genome are two operons that encode high oxygen affinity oxidase types: the cytochrome bd (ubiquinol oxidase) oxidases and the cytochrome cbb3 type. A cytochrome cbb3 oxidase that functions in vitro colonizes anoxic tissues (maximal effect during microaerobiosis). During intracellular multiplication, cytochrome bd oxidase is expressed, allowing cells to adapt to the replicative niche by reducing free radicals' production and eliminated the mechanism of cellular detoxification (Endley et al. 2001; Loisel-Meyer et al. 2005).

The Alkyl Hydroperoxide Reductase Enzyme (AhpC, AhpD)

These enzymes AhpC, AhpD protect cells from oxygen radicals and reactive nitrogen. One promoter control both AhpC and AhpD in an operon. The mutants of AhpC are more sensitive to peroxide killing and spontaneous mutation (Głowacka et al., 2018)

The Nitric Oxide Reductase (NorD) Enzyme

Brucella can use nitric oxide (NO) that infected macrophages produce. There are four types of NorD enzymes in Brucella: the nitrite reductase (Nir), the nitric oxide reductase (Nor), the nitrate reductase (Nar), and the nitrous oxide reductase (Nos), also known as the nitrogen island. When oxygen inside the cell is insufficient the Nitrate is reduced to dinitrogen gas by bacteria, allowing them to respire nitrate. Brucella is able to produce these enzymes to protect itself from low oxygen conditions within the macrophage (Loisel-Meyer et al. 2006)

BvfA (Brucella virulence factor A)

Brucella-specific periplasmic protein; there are no homologous sequences in GenBank. In macrophages, phagosome induces bvfA expression. Possibly, this protein plays a role in establishing the intracellular replication niche. It has not been precisely identified how BvfA functions (Hamdy and Zaki 2018)

The Base Excision Repair (BER)

DNA base excision repair is performed by XthA, a gene that encodes exonuclease III. The Brucella genome contains two different XthA sequences (xthA-1 and xthA-2), this enzyme plays an important role in the prevention of oxidative damage. xthA-1 mutations cause the cells to become more susceptible to reactive oxygen species (ROS) (Poncin et al. 2019).

BvrR/BvrS System

There are two identified open reading frames (ORF) : (*bvrR* and *bvrS*) of the Brucella genomic. The BvrR gene encodes the BvrR protein (237 amino acids) while th BvrS gen encodes the BvrS protein (601 amino acids). (Viadas et al. 2010). BvrR shows similarities to response regulators because its N-terminal domain contains highly conserved amino acids: aspartic (pos: 14, 15, 58) and lysine (pos: 107). A high degree of similarity was found between the C-terminus sequence and OmpR family, so this protein belongs to this family. There are three highly conserved domains in the protein: the N-terminal sensing domain, the periplasmic domain combined with the transmembrane component, the cytoplasmic domain containing histidine residues, and the C-terminal ATP-binding domain (Bialer et al. 2020).
In Brucella, BvrR and BvrS are virulence factors that are best characterized; mutants cannot invade, prevent of phagosomes fuse with lysosomes, or replicate inside cells (Bialer et al. 2019) BvrR / BvrS system are regulate multiple genes. These proteins influence the transcription of membrane proteins: Omp3a (Omp25a) or Omp3b (Omp22) and influence other non-protein membrane molecules and thus, functional and structural membrane homeostasis (Zhang et al. 2017). The BvrR/bvrS mutants show structural changes in LPS, but the O-chains remain intact. Since they are unable to activate GTPase (Cdc42) before entering cells, these mutants persist extracellularly and, consequently, do not infect the cells. The BvrR/BvrS fusion proteins play a role in lysosome fusion and intracellular trafficking (Guzmán-Verri et al. 2001)

Pathogenesis

Both animals and humans are affected by Brucellosis because the same event takes place when a bacterium interacts with its host cell. Brucella can multiply inside macrophages and survive in them, which makes it pathogenic (Liu 2015)
The severity of Brucellosis depends on the number and virulence of the infecting organisms, as well as the host's susceptibility. Proliferation is the goal of Brucella pathogens in the cell (de Figueiredo et al. 2015). Brucella species, as well as other intracellular pathogens, require adhesion, invasion,

establishment, and dissemination to establish themselves and spread throughout the host (Bialer et al., 2020). The smooth and rough strains of Brucella species are both capable of invading epithelial cells, enabling infection through mucosal surfaces, and are both capable of invading phagocytic and non-phagocytic cells (López-Santiago et al. 2019)

It replicates in macrophages, dendritic cells, and placental trophoblasts, showing a strong tissue tropism. Despite this, the pathogen can replicate in many types of mammalian cells, including microglia, fibroblasts, epithelial cells, and endothelial cells. Brucella's main targets are macrophages, dendritic cells (DCs), and trophoblasts (Ahmed et al. 2016)

Additionally, Brucella has the ability to multiply in epithelioid cells (HeLa) and murine fibroblasts (NIH3T3). Brucella invasion, survival, and replication were studied in great detail in phagocytes but not very well in trophoblasts (Kim 2015)

Invasion of the Cell by Brucella

Animal oral mucosa and M cells from mucosa-associated lymphoid tissue of the human digestive tract are the primary entry points of Brucella species (Paixão et al. 2009)

A professional phagocyte (macrophages and DC cells) engulfs a bacterium when it passes through the mucosal epithelium. Following infection, brucellae remain in nonphagocytic cells for up to seventy two hours, then cross the epithelial barrier and enter phagocytic cells. In this initial phase, 10 percent of the bacteria will survive. By breeding and spreading in macrophages, pathogens are able to escape the immune response of the host; therefore, they are able to multiply and invade other tissues. There is a zipper-like mechanism by which Brucella strains invade host cells (Stranahan and Arenas-Gamboa 2021)

Brucella species are spread by the lymphoid tissue of the region, then localized and produced in lymph nodes, before being transported via the bloodstream to parenchymatous organs and tissues. The localization of the bacteria occurs primarily in joint reproductive organs and related glands

During the third trimester of animal pregnancy, there is a high concentration of erythritol, which supports the growth of intra-trophoblastic Brucella, which compromises placental integrity and causes fetal infection, resulting in abortion or weak offspring. Brucella causes acute or chronic infections of the reproductive tract that lead to abortions or severe reproductive diseases (González-Espinoza et al. 2021)

Opsonized organisms are internalized via complement and Fc receptors while Non-opsonized Brucella organisms are internalized via lectin or fibronectin receptors. Pathogens attach to sialic acid residues and sulfated residues on epithelial cells when they come into contact with them (Moreno and Barquero-Calvo 2020).

To penetrate epithelial cells, actin polymerization is necessary. Brucella abortus activates Rho, Rac, and Cdc42 GTPases by adhering to the cell surface. These proteins regulate the cytoskeletal system and regulate the internalization of parasitic bacteria. The only GTPase activated by *Br. abortus* in response to nonphagocytic cells is Cdc42. Other GTPases (Rho or Rac) are believed to be indirectly activated by their inhibition, which prevents invasion into host cells. Additionally, cGMP, PIP3-kinase, MAP-kinase, and tyrosine kinase are involved in adhesion between bacteria and host cells as second messengers (Kim 2015).

Adhesion

Activation of small GTPases plays a role in adhesion to macrophage surfaces and polymerization of F-actin (transient and rapid F-actin accumulation). A protein called Annexin I, implicated in membrane fusion, is also involved in the early stages of adhesion (Kusumawati et al. 2000). The microdomains (lipid rafts), found on the cell membrane of macrophages, are also responsible for bacterial internalization. These structures facilitate the intracellular trafficking of Brucella (Xavier et al., 2014). Through lipid rafts, human monocytes and murine macrophages achieve internalization of nonopsonized Brucella strains. For this process to take place, TLR4 and PI3K must be activated. However, in human dendritic cells, however, lipid rafts are only partially responsible for this process. Strains of Brucella that lack O-polysaccharides in LPS (R-LPS) cannot penetrate eukaryotic cells and are therefore eliminated by macrophages. These lipid rafts contain cholesterol, glycosylphosphatidylinositol (GPI), and ganglioside GM1. Several proteins associated with lipid rafts: GPI and GM1, as well as cholesterol, inoculate with Brucella-contained macropinosomes and facilitate internalization with macrophages.

Intracellular Trafficking

Generally, intracellular trafficking among professional phagocytes and non-professional phagocytes is not remarkably different (Arenas et al. 2000). The bacteria attach to an early endosomal network called a Brucella Containing Vacuole (BCV) after invasion. Early endosomal antigen 1 (EEA1) and GTP-binding protein (Rab5) are markers for this compartment (de Figueiredo et al. 2015)

β-1,2-glucan regulate BCV maturation in macrophages and epithelial cells, also contributes to the formation of cholesterol-rich lipid rafts on the surface of Brucella Containing Vacuole membranes. It takes about 10 minutes to interact with the early endocytic network (Starr et al. 2012). Acidification of BCV at this stage leads to changes in bacterial gene expression and allows intracellular survival of bacteria. By preventing fusion of lysosomes with β-glucans and LPS occurrence, Brucella Containing Vacuole does not react with late endosomes. It indicates interaction with endosomes and lysosomes is required when early BCV transforms into intermediate BCV loaded with LAMP1 and Rab-7 (late endosomal/lysosomal markers) (Jiao et al. 2021).

A Rab-7 effector called Rab-interacting lysosomal protein (RILP) is responsible for acquiring BCV during this process. The interaction between late endosomes/lysosomes and BCV is transitional and controlled (Cantalupo et al. 2001). Subsequently, BCV is acidified and acidic contingent bacterial factors, such as virB, are expressed, while cathepsin D action is prevented. The virB operon encodes the type IV secretion (T4SS), which is required for transporting intracellular materials from the autophagosome to the endoplasmic reticulum in the cell (Ke et al. 2015)

Brucella bacteria are present inside multi-membranous autophagosomes with LAMP1 and Sec61β (calreculin) within an hour of internalization, It occurs only in epithelial cells and is also known as a late BCV. LAMP1 function is unknown, but it appears to contribute to bacterial survival within the cell. Calnexin, Calreticulin, and Sec61β are endoplasmic reticulum markers acquired by BCV during intercellular trafficking. However, BCV loses its ability to make LAMP-1 during this

phase. Bellaire et al. (2005) reported that this protein is detected always in the large vacuoles of human monocytes, where Brucella opsonized reproduces. Endoplasmic reticulum is the only suitable compartment for Brucella multiplication. However, the BCV-ER connection remains unclear. Trafficking of Golgi-bound vesicles to the ER is controlled by Coat Protein Complex I (COPI) and PKCI. Brucella replication in the endoplasmic reticulum is influenced by a variety of factors, including Coat Protein Complex I, GTPase (Rab2), glyceraldehyde-3-phosphate dehydrogenase, and PKCI (Fugier et al. 2009)

Diagnosis

Many Brucella species have been isolated using Thayer's, Martin's, and Farrell's as enrichment and selective media, and after 4 to 6 days the colonies growth when of incubated at 37 °C. However, at 28 °C, they grow slowly and poorly. Additionally, these bacteria can grow with or without 10% carbon dioxide, but they grow better without CO_2 on serum dextrose agar (Yagupsky et al. 2020). Bacteria can be cultured in many media such as Tryptone soya, Triptic soya, Triptcase soya and Bacto tryptose. In addition, Biphase Castaneda medium used for blood and body fluid culture (Yagupsky, 2015). The liquid Castaneda medium contains between 1 and 2% sodium citrate. An antibody level in serum is measured as part of a serological test to detect infection. Brucella infection in the 1st week is characterized by IgM titers, whereas IgG titers dominate in the 2ed week. After two months, both antibodies IgA and IgG are at their peak; excessive IgG levels may indicate mistreatment (Yagupsky et al. 2020)

In serology, enzyme-linked immunosorbent assays (ELISAs) and serum agglutination tests (SATs) are useful tests for diagnosing Brucellosis (Hajia et al. 2013)

Enzyme-linked immunosorbent assays detects antibodies in serum against the S-LPS antigen (Asfaw et al. 2015). However, through molecular techniques such as classical PCR, RT PCR can be used to detect Brucellosis by different pair of primers. Among genes used for identifying Brucella species are the omp2 gene (primer: JPF/JPR), rRNA sequences from 16S (primers: F4/R2), and BCSP 31 (primers: B4/B5), (Yu and Nielsen 2010).

Treatment of Brucellosis

Brucella vaccines for humans are not yet available, but there are many Brucella vaccines for livestock (Lalsiamthara and Lee 2017). Live, attenuated vaccinations that lack virulence components (e.g., the Live *Br.abortus* vaccine strain RB51, the Rev-1 Live *Br.melitensis* vaccine strain Rev-1, and the Live *Br.abortus* vaccine strain 19), yet still have residual pathogenicity (Aragón-Aranda et al. 2020). The use of subunit vaccinations has been shown to be generally safe and cause fewer complications than live immunizations. The immune system is stimulated by purified proteins or DNA, so they do not induce infection. In addition to developing vaccines for animals, researchers are also finding new ways to prevent human disease (Yang et al. 2013).

There are several therapies available to treat Brucellosis, which rarely causes death. In order to treat Brucellosis successfully, an antibiotic must penetrate macrophages and be active in acidic environments. However, does not respond to single antibiotic therapy, leading to relapses.

As with single agents such as oxytetracycline, rifampin, or doxycycline, the rate of relapse with these therapies can reach 9–25% and prolonging the therapy does not have any significant effect. In 30% of cases, trimethoprim-sulfamethoxazole causes relapses, while ciprofloxacin causes relapses in 83% (Gültekin et al. 2021).

The combination of two antibiotics is more effective than monotherapy in treating Brucella-induced infections. In the WHO guidelines (1986), doxycycline and rifampicin should be combined for six weeks and then switched to tetracycline and streptomycin (Alavi and Alavi, 2013). A number of antibiotic combinations and chemotherapy are currently available to treat Brucellosis, including fluoroquinolones, streptomycin with doxycycline (SD), and co-trimoxazole with rifampicin (RCTM) (Colmenero et al. 1994; Hosseini et al. 2019)

Brucellosis treatment by using doxycycline (SD) with streptomycin resulted in a relapse rate of 4.8% and a failure rate of 7.4% (Solís García del Pozo and Solera 2012). Children treated with doxycycline and gentamicin (DG) fail therapy on average by 5.2%, with relapse rates of 5.9% (Alavi and Alavi 2013) . Children treated with co-trimoxazole and rifampicin (RCTM) fail therapy on average by 0% to 16.4% with relapse rates of 3.1% to 10% (Alavi and Alavi 2013).

According to three clinical trials, the relapse rates varied from 3.2 -26 % (average 11.4%) and failure rates ranged from 3.2 - 26% (average 12.2%) with ciprofloxacin or ofloxacin and doxycycline, co-trimoxazole, and rifampicin used (Alavi and Alavi 2013).

Three clinical trials used doxycycline, rifampicin, and aminoglycosides. No evidence exists to support the superiority of triple-drug treatments over two-drug treatments. Triple drug therapy prevents relapses better, but is not effective for treating short-term symptoms, according to (Solís García del Pozo and Solera 2012) . It can be effective to administer triple therapy for eight weeks in arthritis or spondylitis cases.

If the condition is chronic or acute, or if endocarditis, spondylitis or arthritis have not developed, doxycycline and aminoglycosides are recommended. For simple condition, or gentamicin, doxycycline or streptomycin, may be recommended (Alavi and Alavi 2013).

A new strategy for treating Brucellosis was developed by (Smith et al. 2013). For BCV to bind to the ER, the endoplasmatic reticulum must be remodelled to alter the ER structure during the host stress response, which is called the Unfolded Protein Response (UPR). Brucella replication can be inhibited by tauroursodeoxycholic acid, a drug that disrupts UPR. A novel mechanism for treating Brucellosis may involve UPR (Smith et al. 2013)

Research has been conducted on the antibrucellosis effects of RGSF-A (ginseng saponin fraction A). Asia considers ginseng (a valued plant) to be a panacea for a variety of diseases. Researchers found that treated cells by RGSF-A inhibits the polymerization of F-actin and the invasion of bacteria into cells, decreased bacterial adhesion and internalization compared to control cells, inhibiting MAPKs (mitogen-activated protein kinases).

The RGSF-A protein enhances *Br.abortus* intracellular trafficking as well as the interaction between Brucella abortus-containing phagosomes and LAMP-1 (Arayan et al. 2015). A transmembrane protein, LAMP-1 controls the fusion of lysosomes with phagosomes, allowing BCPs to connect with lysosomes and eliminate bacteria. According to (Huy et al. 2017), RGSF-A has been shown to be the most effective

inhibitor of Brucellosis through its component of ginsenoside-panaxadiol saponin. Furthermore, several plants are effective against brucellosis, that contain bioactive elements (flavonoids, flavones, tannins, and anthocyanins), these plants including Teucrium polium, Scophularia deserti, Alhagi, Eucalyptus, garlic and roots of barberry (Alizadeh et al. 2018).

Conclusions

Brucella is a bacterium that is particularly hazardous to domestic animals, causing widespread infections and, as a result, enormous economic loss. Furthermore, people who work with animals that are infected, such as farmers, veterinarians, or laboratory technicians, are susceptible to contracting the disease. In humans, Brucellosis causes vague symptoms, so it is impossible to estimate how many people are infected. Brucella is a curious etiological agent that lacks traditional virulence determinants. Infection is a complicated process with many unexplained problems. As a result, more research is needed on infection pathways is necessary.

REFERENCES

Ahmed W et al., 2016. Establishment of Chronic Infection: Brucella's Stealth Strategy. Frontiers in Cellular and Infection Microbiology 6:30.

Alavi SM and Alavi L, 2013. Treatment of brucellosis: a systematic review of studies in recent twenty years. Caspian Journal of Internal Medicine 4:636.

Alizadeh M et al., 2018. Brucellosis: Pathophysiology and new promising treatments with medicinal plants and natural antioxidants. Asian Pacific Journal of Tropical Medicine 11:597-608.

Alton GG and Forsyth JRL, 1996. Brucella. Molecular Medical Microbiology: Second Edition 3:1781-1788.

Alton GG et al., 1989. Diagnosis of bovine brucellosis: Principles, practice and problems. Surveillance 16:3-6.

Aragón-Aranda B et al., 2020. Development of attenuated live vaccine candidates against swine brucellosis in a non-zoonotic B. suis biovar 2 background. Veterinary Research 51:1-14.

Araj GF, 2010. Update on laboratory diagnosis of human brucellosis. International Journal of Antimicrobial Agents 36: S12-S17.

Arayan LT et al., 2015. The effects of red ginseng saponin fraction-A (RGSF-A) on phagocytosis and intracellular signaling in Brucella abortus infected RAW 264.7 cells. FEMS Microbiology Letters 362.

Arenas GN et al., 2000. Intracellular Trafficking of Brucella abortus in J774 Macrophages. Infection and Immunity 68:4255.

Asfaw M et al., 2015. A Review on Diagnostic Methods of Brucellosis. Journal of Veterinary Science and Technology 07.

Assenga JA et al., 2015. Epidemiology of Brucella infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. BMC Veterinary Research 11:1-11.

Ay N et al., 2016. Pulmonary Involvement in Brucellosis, a Rare Complication of Renal Transplant: Case Report and Brief Review. Experimental and Clinical Transplantation : Official Journal of the Middle East Society for Organ Transplantation 16:757-760.

Balloux F and van Dorp L, 2017. Q&A: What are pathogens, and what have they done to and for us? BMC Biology 15.

Von Bargen K et al., 2012. Internal affairs: investigating the Brucella intracellular lifestyle. FEMS Microbiology Review 36:533-562.

Bellaire BH et al., 2005. Opsonized virulent Brucella abortus replicates within nonacidic, endoplasmic reticulum-negative, LAMP-1-positive phagosomes in human monocytes. Infection and Immunity 73:3702-3713.

Bialer MG et al., 2019. MapB, the Brucella suis TamB homologue, is involved in cell envelope biogenesis, cell division and virulence. Scientific Reports 9:1-18.

Bialer MG et al., 2020. Adhesins of Brucella: Their Roles in the Interaction with the Host. Pathogens 9:1-20.

Boschiroli ML et al., 2002. Type IV secretion and Brucella virulence. Veterinary Microbiology 90:341-348.

Boussetta M, 1991. Laboratory diagnosis of animal brucellosis. Archive Institute Pasteur de Tunis 68:285-293.

Cantalupo G et al., 2001. Rab-interacting lysosomal protein (RILP): the Rab7 effector required for transport to lysosomes. The EMBO Journal 20:683.

Cardoso PG et al., 2006. Brucella spp noncanonical LPS: structure, biosynthesis, and interaction with host immune system. Microbial Cell Factories 5:13.

Celli J et al., 2003. Brucella Evades Macrophage Killing via VirB-dependent Sustained Interactions with the Endoplasmic Reticulum. Journal of Experimental Medicine 198:545- 556.

Christopher SL et al., 2010. Brucellosis: review on the recent trends in pathogenicity and laboratory diagnosis. Journal of Laboratory Physicians 2:055-060.

Colmenero JD et al., 1994. Possible implications of doxycycline-rifampin interaction for treatment of brucellosis. Antimicrobial Agents and Chemotherapy 38:2798.

Conde-Álvarez R et al., 2012. The Lipopolysaccharide Core of Brucella abortus Acts as a Shield Against Innate Immunity Recognition. PLOS Pathogens 8:e1002675.

Corbel MJ, 1997. Brucellosis: an overview. Emerg Infect Dis 3:213- 221.

Al Dahouk S et al., 2003. Laboratory-based diagnosis of brucellosis - A review of the literature Part I: Techniques for direct detection and identification of Brucella spp. Clinical Laboratory 49:487-505.

Al Dahouk S et al., 2010. Differential phenotyping of Brucella species using a newly developed semi-automated metabolic system. BMC Microbiology 10:1-12.

Elderbrook M et al., 2019. Seroprevalence and risk factors of Brucella ovis in domestic sheep in Wyoming, USA. BMC Veterinary Research 15:1-12.

Endley S et al., 2001. Interruption of the cydB locus in Brucella abortus attenuates intracellular survival and virulence in the mouse model of infection. Journal of Bacteriology 183:2454-2462.

De Figueiredo P et al., 2015. Pathogenesis and Immunobiology of Brucellosis: Review of Brucella–Host Interactions. The American Journal of Pathology 185:1505-1517.

Fouskis I et al., 2018. The epidemiology of Brucellosis in Greece, 2007–2012: a 'One Health' approach. Transactions of The Royal Society of Tropical Medicine and Hygiene 112:124-135.

Franc KA et al., 2018. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC Public Health 18.

Fugier E et al., 2009. The glyceraldehyde-3-phosphate dehydrogenase and the small GTPase Rab 2 are crucial for Brucella replication. PLoS Pathog 5.

Gee JM et al., 2004. Role of catalase in the virulence of Brucella melitensis in pregnant goats. Veterinary Microbiology 102:111-115.

Głowacka P et al., 2018. Brucella – Virulence Factors, Pathogenesis and Treatment. Polish Journal of Microbiology 67:151.

Godfroid J et al., 2013. A "One Health" surveillance and control of brucellosis in developing countries: Moving away from improvisation. Comparative Immunology, Microbiology and Infectious Diseases 36:241-248.

González-Espinoza G et al., 2021. Brucella: Reservoirs and Niches in Animals and Humans. Pathogens 10:186

Gültekin E et al., 2021. Investigation of antibiotic susceptibilities of Brucella Strains isolated from various clinical samples in eastern Turkey. European Journal of Medical Research 26:1-6.

Guzmán-Verri C et al., 2001. GTPases of the Rho subfamily are required for Brucella abortus internalization in nonprofessional phagocytes: direct activation of Cdc42. Journal of Biological Chemistry 276: 44435-44443.

Gwida M et al., 2010. Brucellosis – Regionally Emerging Zoonotic Disease? Croatian Medical Journal 51:289.

Hajia M et al., 2013. Comparison of Methods for Diagnosing Brucellosis. Laboratory Medicine 44:29-33.

Hamdy MER and Zaki HM, 2018. Detection of virulence-associated genes in brucella melitensis biovar 3, the prevalent field strain in different animal species in Egypt. Open Veterinary Journal 8:112-117.

Hasanuzzaman M et al., 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants 9:1-52.

Hosseini SM et al., 2019. Doxycycline-encapsulated solid lipid nanoparticles for the enhanced antibacterial potential to treat the chronic brucellosis and preventing its relapse: In vivo study. Annals of Clinical Microbiology and Antimicrobials 18:1-10.

Huy TXN et al., 2017. Intracellular Trafficking Modulation by Ginsenoside Rg3 Inhibits Brucella abortus Uptake and Intracellular Survival within RAW 264.7 Cells. Journal of Microbiology an Biotechnology 27:616-623.

Jiao H et al., 2021. The Mechanism of Facultative Intracellular Parasitism of Brucella. International Journal of Molecular Sciences 22.

Ke Y et al., 2015. Type IV secretion system of Brucella spp. and its effectors. Frontiers in Cellular and Infection Microbiology 5:72.

Khurana SK et al., 2021. Bovine brucellosis – a comprehensive review. The Veterinary Quarterly 41: 61.

Kim S, 2015. The Interaction Between Brucella and the Host Cell in Phagocytosis. Updates on Brucellosis.

Köse Ş et al., 2005. Identification of Brucella species isolated from proven brucellosis patients in Izmir, Turkey. Journal of Basic Microbiology 45:323-327.

Kusumawati A et al., 2000. Early events and implication of F-actin and annexin I associated structures in the phagocytic uptake of Brucella suis by the J-774A.1 murine cell line and human monocytes. Microbial Pathogensis 28:343-352.

Lalsiamthara J and Lee JH, 2017. Development and trial of vaccines against Brucella. Journal of Veterinary Science 18:281.

Lapaque N et al., 2005. Brucella lipopolysaccharide acts as a virulence factor. Current Opinion in Microbiology 8:60-66.

Ledwaba MB et al., 2020. Investigating selective media for optimal isolation of Brucella spp. in South Africa. The Onderstepoort Journal of Veterinary Research 87.

Liu D, 2015. Brucella. Molecular Medical Microbiology: Second Edition 3:1781-1788.

Loisel-Meyer S et al., 2005. Differential use of the two high-oxygen-affinity terminal oxidases of Brucella suis for in vitro and intramacrophagic multiplication. Infection and Immunity 73:7768-7771.

Loisel-Meyer S et al., 2006. Requirement of norD for Brucella suis Virulence in a Murine Model of In Vitro and In Vivo Infection. Infection and Immunity 74:1973.

López-Santiago R et al., 2019. Immune response to mucosal brucella infection. Frontiers in Immunology 10:1759.

Makita K et al., 2011. Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. BMC Veterinary Research 7:60.

Maldonado RF et al., 2016. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiology Reviews 40:480.

McDermott J et al., 2013. Economics of brucellosis impact and control in low-income countries. Review Scientific Technique (International Office o Epizootics) 32:249-261.

de Miguel MJ et al., 2011. Development of a selective culture medium for primary isolation of the main Brucella Species. Journal of Clinical Microbiology 49:1458-1463.

Moreno E and Barquero-Calvo E, 2020. The Role of Neutrophils in Brucellosis. Microbiology and Molecular Biology Reviews: MMBR 84.

Moreno E et al., 1981. Biological activities of Brucella abortus lipopolysaccharides. Infection and Immunity 31:362-370.

Morgan WJB, 1990. Techniques for the Brucellosis laboratory: G. G. Alton, L. M. Jones, R. D. Angus & J. M. Verger Versailles Cedex: INRA Publications. 1988. 192pp. Ff 195. The British Veterinary Journal 146:188.

Muma JB et al., 2007. Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia. Preventive Veterinary Medicine 80:306-317.

Xavier M et al., 2014. Pathogenesis of Brucella spp. The Open Veterinary Science Journal 4:109-118.

Ndegwa EN et al., 2001. Prevalence of microorganisms associated with udder infections in dairy goats on small-scale farms in Kenya. Journal of the South African Veterinary Association 72:97-98.

Padilla Poester F et al., 2014. Diagnosis of Brucellosis. The Open Veterinary Science Journal 4:46-60.

Paixão TA et al., 2009. Establishment of Systemic Brucella melitensis Infection through the Digestive Tract Requires Urease, the Type IV Secretion System, and Lipopolysaccharide O Antigen. Infection and Immunity 77:4197.

Poncin K et al., 2019. Occurrence and repair of alkylating stress in the intracellular pathogen Brucella abortus. Nature Communications 10:1-13.

Rahman MS et al., 2006. A Short History of Brucellosis: Special Emphasis in Bangladesh. Bangladesh Journal of Veterinary Medicine 4:1-6.

Roset MS et al., 2014. Brucella Cyclic β-1,2-Glucan Plays a Critical Role in the Induction of Splenomegaly in Mice. PLoS One 9: e101279.

Sauret JM and Vilissova N, 2002. Human brucellosis. The Journal of the American Board of Family Medicine 15.

Seleem MN et al., 2008. Brucella: a pathogen without classic virulence genes. Veterinary Microbiology 129:1-14.

Sieira R et al., 2010. Metabolic control of virulence genes in Brucella abortus: HutC coordinates virB expression and the histidine utilization pathway by direct binding to both promoters. Journal of Bacteriology 192:217-224.

Smith JA et al., 2013. Brucella induces an unfolded protein response via TcpB that supports intracellular replication in macrophages. PLoS Pathogens 9:1-12.

Solís García del Pozo J and Solera J, 2012. Systematic Review and Meta-Analysis of Randomized Clinical Trials in the Treatment of Human Brucellosis. PLoS One 7.

Starr T et al., 2012. Selective subversion of autophagy complexes facilitates completion of the Brucella intracellular cycle. Cell Host and Microbe 11:33-45.

Stranahan LW and Arenas-Gamboa AM, 2021. When the Going Gets Rough: The Significance of Brucella Lipopolysaccharide Phenotype in Host–Pathogen Interactions. Frontiers in Microbiology 12:1956.

Tekle M et al., 2019. Isolation and identification of Brucella melitensis using bacteriological and molecular tools from aborted goats in the Afar region of north-eastern Ethiopia. BMC Microbiology 19:1-6.

Viadas C et al., 2010. Transcriptome Analysis of the Brucella abortus BvrR/BvrS Two-Component Regulatory System. PLoS One 5:e10216.

Xiong X et al., 2021. The VirB System Plays a Crucial Role in Brucella Intracellular Infection. International Journal of Molecular Sciences 22:13637.

Yagupsky P, 2015. Blood Cultures for the Diagnosis of Human Brucellosis. Updates on Brucellosis.

Yagupsky P et al., 2020. Laboratory Diagnosis of Human Brucellosis. Clinical Microbiology Reviews 33.

Yang X et al., 2013. Progress in Brucella vaccine development. Frontiers in Biology (Beijing) 8:60-77.

Yu WL and Nielsen K, 2010. Review of Detection of Brucella sp. by Polymerase Chain Reaction. Croatian Medical Journal 51:306.

Zhang J et al., 2017. Outer membrane protein 25 of Brucella activates mitogen-activated protein kinase signal pathway in human trophoblast cells. Frontiers in Veterinary Science 4:197.

Zhao Y et al., 2015. Prevalence and pathogens of subclinical mastitis in dairy goats in China. Tropical Animal Health and Production 47:429-435.