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Pathogenesis, clinical manifestations, treatment, and prevention of brucellosis in humans and animals

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ABSTRACT

Brucella genus is a gram-negative coccobacillus that causes a zoonotic disease called brucellosis in humans and animals. Brucellosis is transmissible, and there are different types of transmission, such as animal-to-human (dairy and meat consumption), human-to-human (placenta, breastfeeding, sexual intercourse, blood transfusion, and bone marrow transplantation), and animal-to-animal (aerosol-containing *Brucella*, placenta, and sexual intercourse). Due to having virulence factors such as type IV secretion system (T4SS), superoxide dismutase enzyme, BvrS/BvrR two-component system, LPS with O chain, and cyclic beta-glucan, *Brucella* can survive and replicate intracellularly and cause disease. The T4SS helps *Brucella* survive intracellularly by sending effectors into the host cell. Fever, excessive sweating, malaise, myalgia, arthralgia, loss of appetite, weight loss, hepatosplenomegaly, spondylitis, and arthritis are the clinical manifestations of brucellosis in humans. In animals, complications such as abortion, weak calf birth, reduced fertility, endometritis, reduced milk production, orchitis, epididymis, and hygroma may be observed. Animals with brucellosis are not treated and are slaughtered. No vaccine against brucellosis has been approved for humans yet. But for animals, S19, Rev.1, S2, RB51, and SR82 vaccines are used for prevention. Development and approval of brucellosis vaccines for humans, wider use of animal vaccines, and timely diagnosis of suspected cases will help to better control brucellosis and prevent economic consequences.

Keywords: Brucellosis, *Brucella*, Pathogenicity, Diagnosis, Treatment.

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1. Introduction

Brucellosis is a zoonotic disease caused by *Brucella* species that is transmitted to humans through contact with animals or their products (1). The disease caused by *Brucellae* has different names that correspond to the person who described this genus (Sir David Bruce [Brucellosis], Bernhard Bang [Bang disease]), or is related to the clinical symptoms (undulant fever) or the area where the outbreak occurred (Malt fever, Mediterranean remittent fever, rock fever of Gibraltar, county fever of Constantinople, fever of Crete). Brucellosis is the most common name (2).

Brucella genus are small, aerobic, non-motile, gram-negative coccobacilli, which, in addition to these characteristics, also have catalase, oxidase, and urease activities (3). Also, *Brucellae* are obligate parasites that survive intracellularly in humans and animals. Molecular studies of *Brucella* have shown that there is only one species of *Brucella* (B. melitensis); however, 12 species have been defined for this genus. Among these identified species, four species—B. melitensis (infecting goats), B. suis (infecting swine), B. abortus (infecting cattle), and B. canis (infecting dogs)—are commonly found in humans and animals. The other species only infect animals (2, 4).

The most common way to transmit brucellosis to humans is by consuming unheated milk (from animals such as cows, goats, sheep, and camels) or its products (cheese, butter, cream, and ice cream). *Brucella* can be concentrated in soft cheese made by adding rennet to sheep's or goat's milk, which is a common source of infection in the Mediterranean and the Middle East. Consumption of contaminated raw meat also has the possibility of *Brucella* transmission, which is the muscle with the lowest concentration; the liver, spleen, kidney, breast, and testicle can have the highest concentration of *Brucella*. Occupational exposure and contact with *Brucella*-infected aerosols among slaughterhouse workers, veterinarians, and laboratory technicians cause brucellosis transmission (1, 5). Other ways of brucellosis transmission are human-to-human transmission through the placenta, breastfeeding, sexual intercourse, blood transfusion, and bone marrow transplantation, which rarely occurs (6). In animals, brucellosis is transmitted through ingestion of contaminated food and water, inhalation of *Brucella*-contaminated aerosols, sexual intercourse, and the placenta (7).

It is estimated that more than 500,000 new cases of human brucellosis are reported annually in the world. In Afghanistan, in 2004, there were 3.8 cases per million people with brucellosis, which seems far from reality (8). Areas whose economies are dependent on livestock products experience substantial economic losses due to the loss of livestock in the event of a brucellosis outbreak (9). In Mexico and Argentina, annual damage to livestock farming due to brucellosis is estimated at US$200 million and US$60 million, respectively (10–12). Mortality due to brucellosis is rare, but the illness and the long convalescence that follow are costly in terms of economics and time. Therefore, in the first step, rapid diagnosis, and in the next step, the treatment of brucellosis using antibiotics, reduce the mentioned costs (1, 10).

1. 2. Pathogenicity

In humans, *Brucella* enters the host through ingestion, inhalation, the mucous membrane, or damaged skin. The bacteria multiply in the lymph nodes and then enter other organs. Enlargement of lymph nodes, liver, and spleen is the result of brucellosis. Once the *Brucella* bacterium passes through the epithelial wall, it is engulfed by phagocytes. In addition to phagocytizing cells, bacteria can also survive and multiply in non-phagocytic cells. However, macrophages and dendritic cells are the main target cells of *Brucella*. When *Brucella* is inside the macrophage, it inhibits the macrophage's

responses, which results in the survival and multiplication of *Brucella* inside the cell. (13). In the following, the factors affecting the pathogenicity of Brucella are described.

Urease: The acidic pH of the stomach is effective in preventing colonization of *Brucella* in the intestine as well as invasion. Factors such as proton pump inhibitors and antacid drugs reduce stomach acidity and prepare the conditions for *Brucella* colonization. Urease is an enzyme that produces ammonia and carbon dioxide by hydrolyzing urea. Through its urease, *Brucella* can tolerate the acidic conditions of the stomach and pass through this place (14).

Brucella-containing vacuole (BCV) and type IV secretion system (T4SS): When *Brucella* enters the endosome of the host cell, it receives several markers such as Rab5, Rab7, and EEA, which lead to the formation of a *Brucella*-containing vacuole (BCV). Then, BCV fuses with the lysosome and forms a phagolysosome by receiving the LAMP marker. In this situation, 90% of *Brucella* are killed by enzymes. The remaining 10% probably survive due to acidification of BCV and stimulation of the operon (virB) encoding the type IV secretion system (T4SS).

BvrS/BvrR system: When the phagolysosome becomes acidic, the two-component BvrS/BvrR system of *Brucella* is activated. The BvrS/BvrR system consists of two parts: one part is the transmembrane histidine kinase sensor called BvrS, and the other part is the response regulator that is placed in the bacterial cytosol. The histidine kinase sensor is autophosphorylated at histidine residues during environmental changes (including acidification). Then the phosphate is transferred to the response regulator, which subsequently changes gene expression and creates suitable conditions for intracellular life. The BvrS/BvrR system plays a role in controlling the structure of LPS and controlling the expression of outer membrane proteins such as Omp22 and Omp25. In addition, this system controls the synthesis of the type IV secretion system (T4SS) through the virB operon. The T4SS helps *Brucella* survive intracellularly by sending effectors into the host cell. (15, 16). Next, BCV fuses with the endoplasmic reticulum, and *Brucella* survives and starts replicating. The same actions are then carried out inside the autophagy-like vacuole, and *Brucella* then leaves the cell to infect other cells (17).

Lipopolysaccharide (LPS): *Brucella* is divided into two groups, smooth and rough, according to the shape of the colony (18). *Brucella* smooth strain has lipopolysaccharide (LPS) with a structure consisting of the polysaccharide O chain, core, and lipid A, while the rough strain does not have the O chain. Except for B. ovis and B. canis, rough strains are less virulent than smooth strains due to the lack of the O chain (19). The lack of the O chain causes the vesicle containing *Brucella* to be transported to the lysosome, which kills the bacteria (18). Although the smooth species is more successful in surviving inside the host cell, the rough species is more effective in infecting monocytes (20).

Brucella LPS interferes with antimicrobial innate immune responses through the inhibition of complement and antibacterial peptide function, as well as inhibiting the synthesis of immune mediators (21). In addition, smooth strains of *Brucella* that have O chains prevent apoptosis in host cells (such as macrophages), which allows the bacteria to survive and continue replicating in sufficient numbers to cause an immune response and disease (22).

Superoxide dismutase (SOD) enzyme: The production of reactive oxygen species (ROS) in macrophages is the primary defense mechanism against the intracellular replication of *Brucella*. To neutralize this mechanism, *Brucella* has the superoxide dismutase (SOD) enzyme with zinc and copper cofactors, which is encoded by the sodC gene. *Brucella* converts superoxide (O2) into hydrogen peroxide (H2O2) using superoxide dismutase, and then catalase and peroxidase complete the detoxification mechanism of this bacterium (23). Cyclic β-1,2-glucans (CβG): A member of the osmoregulated periplasmic glucans (OPGs)

family is cyclic β-1,2-glucans (CβG), which is produced in bacteria such as Brucella. The CβG backbone consists of 17 to 25 glucose residues. The function of CβG is different from the rest of the OPG family, as it interacts with the phagosome membrane after its secretion by *Brucella*. The CβG of *Brucella*, through interaction with the lipid raft of the host cell (macrophage and epithelial cell), causing interference in the path of phagosome maturation and preventing the fusion of the phagosome with the lysosome, causes survival and replication of bacteria inside the host cell (24).

In animals, the incubation period for brucellosis can range from two weeks to months. The most important complication of brucellosis in animals is related to the reproductive system. In a female animal with brucellosis, complications such as abortion (30–80%), weak calf birth, reduced fertility, fetal membrane retention, endometritis, and reduced milk production may occur. Orchitis, epididymitis, and hygroma have also been reported in male animals with brucellosis (25).

1.3. Diagnostic methods

Culture-based diagnosis is the gold standard and definitive diagnosis. Samples such as blood, bone marrow, CSF, joint fluid, urine, and other tissues are cultured. Other diagnostic methods are indirect, including the Rose Bengal test (RBT), tube standard agglutination test (SAT), 2-mercaptoethanol test, Coombs test, and ELISA. RBT and SAT are based on the detection of agglutination (an antibody reaction of the patient's serum with *Brucella* S-LPS antigen related to the kit), which is performed on the slide and inside the tube, respectively. RBT is a screening test; therefore, if the RBT is positive, it is necessary to perform the SAT for confirmation (29).

1.4. Treatment

If the person with brucellosis is not pregnant, is more than 8 years old, and does not have complications such as endocarditis, spondylitis, or neurological complications, one of the following treatments should be prescribed for them: 1) Doxycycline 200 mg/day orally for 6 weeks + Streptomycin 1 g/day IM or IV for the first 2-3 weeks; 2) Doxycycline 200 mg/day orally for 6 weeks + Gentamicin 3-5 mg/kg/day IM or IV for 1-2 weeks; 3) Doxycycline 200 mg/day orally for 6 weeks + Rifampin 600-900 mg/day orally for 6 weeks.

To treat *brucella* endocarditis, a combination of three or four drugs, including aminoglycoside (1 month), doxycycline, rifampin, and sometimes TMP-SMX (3–15 months for three drugs), is used. Both rifampin and TMP-SMX are used to treat children under 8 years old, and one or both drugs are used for pregnant women for 6 weeks. It should be noted that TMP-SMX should only be prescribed between the 13th and 36th weeks of pregnancy because its use before this time causes teratogenicity, and after that, it causes kernicterus (30). If brucellosis is detected in animals, the animal is slaughtered under safety conditions to prevent transmission to the herd (1).

1.5. Vaccines

So far, no vaccine against brucellosis has been approved for human use. Instead, B. abortus vaccine strain S19 (S19), B. melitensis Rev.1 vaccine (Rev.1), B. suis vaccine strain S2 (S2), B. abortus RB51 vaccine (RB51), and B. abortus SR82 vaccine (SR82) vaccines are used for animals to prevent human brucellosis. Despite the possibility of complications such as abortion in pregnant animals, these vaccines play a major role in preventing brucellosis worldwide. The live attenuated vaccines S19, Rev.1, and S2 are related to smooth strains. Also, RB51 and SR82 are attenuated vaccines obtained from the B. abortus strains (rough). The first brucellosis vaccine used is S19, which is widely used for cattle vaccination in many countries. This vaccine consists of the whole organism of B. abortus strain 19, with no adjuvant added. Providing high immunity in cattle and interference with serological tests are

advantages and disadvantages of the S19 vaccine, respectively (31).

Rev.1 vaccine is a live attenuated vaccine of streptomycin-resistant strain B melitensis, which is used for female animals aged 2 to 6 months. This vaccine, while being immunogenic, has the potential to infect humans by consuming the product of the vaccinated animal. (32) S2, RB51, and SR82 vaccines are composed of the whole organism and do not contain adjuvants. S2 vaccine is used orally for swine, sheep, goats, and cows in China and SR82 in Russia and its neighboring countries. Since RB51 is less pathogenic for humans than S19, this vaccine is used in many countries (31).

2. Conclusion

Brucella is a zoonosis because it causes disease in both humans and animals. Cattle with brucellosis are usually slaughtered after diagnosis, and their products, including dairy and meat, cannot be used. Considering this issue and the characteristic of brucellosis being contagious in herds, *brucella* should be considered an economically damaging agent. Due to the intracellular survival and replication of *Brucella*, brucellosis may become chronic or the treatment may fail and relapse, resulting in economic consequences. Therefore, the development and approval of human brucellosis vaccines, the wider use of animal vaccines, and the diagnosis of suspected cases of the disease as quickly as possible, especially in endemic areas, seem essential.

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Reference

1. Corbel MJ. Brucellosis in humans and animals: World Health Organization; 2006.
2. Murray PR, Rosenthal KS, Pfaller MA. Medical microbiology: Elsevier Health Sciences; 2020.
3. Percin D. Microbiology of brucella. Recent patents on anti-infective drug discovery. 2013;8(1):13-7. doi:10.2174/1574891X11308010004
4. Brooks G, Carroll K, Butel J, Morse S, Mietzner T. Medical Microbiology. Jawetz, Melnick and Adelbergs. McGraw-Hill Companies; 2010.
5. Mantur BG, Biradar MS, Bidri RC, Mulimani MS, K V, Kariholu P, et al. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. Journal of medical microbiology. 2006;55(Pt 7):897-903. doi:10.1099/jmm.0.46097-0
6. Tuon FF, Gondolfo RB, Cerchiari N. Human-to-human transmission of Brucella - a systematic review. Tropical medicine & international health : TM & IH. 2017;22(5):539-46. doi:10.1111/tmi.12856
7. Islam MA, Khatun MM, Werre SR, Sriranganathan N, Boyle SM. A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. Veterinary microbiology. 2013;166(3-4):317-26. doi:10.1016/j.vetmic.2013.06.014
8. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. The Lancet Infectious diseases. 2006;6(2):91-9. doi:10.1016/s1473-3099(06)70382-6
9. Pappas G, Panagopoulou P, Christou L, Akritidis N. Brucella as a biological weapon. Cellular and molecular life sciences : CMLS. 2006;63(19-20):2229-36. doi:10.1007/s00018-006-6311-4
10. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. The Lancet Infectious diseases. 2007;7(12):775-86. doi:10.1016/s1473-3099(07)70286-4
11. Luna-Martínez JE, Mejía-Terán C. Brucellosis in Mexico: current status and trends. Veterinary microbiology. 2002;90(1-4):19-30. doi:10.1016/s0378-1135(02)00241-9
12. Samartino LE. Brucellosis in Argentina. Veterinary microbiology. 2002;90(1-4):71-80. doi:10.1016/s0378-1135(02)00247-x
13. Głowacka P, Żakowska D, Naylor K, Niemcewicz M, Bielawska-Drózd A. Brucella - Virulence Factors, Pathogenesis and Treatment. Polish journal of microbiology. 2018;67(2):151-61. doi:10.21307/pjm-2018-029
14. Sangari FJ, Seoane A, Rodríguez MC, Agüero J, García Lobo JM. Characterization of the urease operon of Brucella abortus and assessment of its role

in virulence of the bacterium. Infect Immun. 2007;75(2):774-80. doi:10.1128/iai.01244-06
15. Ramírez-González EA, Moreno-Lafont MC, Méndez-Tenorio A, Cancino-Díaz ME, Estrada-García I, López-Santiago R. Prediction of Structure and Molecular Interaction with DNA of BvrR, a Virulence-Associated Regulatory Protein of Brucella. Molecules (Basel, Switzerland). 2019;24(17). doi:10.3390/molecules24173137
16. Martínez-Núñez C, Altamirano-Silva P, Alvarado-Guillén F, Moreno E, Guzmán-Verri C, Chaves-Olarte E. The two-component system BvrR/BvrS regulates the expression of the type IV secretion system VirB in Brucella abortus. J Bacteriol. 2010;192(21):5603-8. doi:10.1128/jb.00567-10
17. Ke Y, Wang Y, Li W, Chen Z. Type IV secretion system of Brucella spp. and its effectors. Frontiers in cellular and infection microbiology. 2015;5. doi:10.3389/fcimb.2015.00072
18. López-Santiago R, Sánchez-Argáez AB, De Alba-Núñez LG, Baltierra-Uribe SL, Moreno-Lafont MC. Immune Response to Mucosal Brucella Infection. Frontiers in Immunology. 2019;10. doi:10.3389/fimmu.2019.01759
19. Guo X, Zeng H, Li M, Xiao Y, Gu G, Song Z, et al. The mechanism of chronic intracellular infection with Brucella spp. Frontiers in cellular and infection microbiology. 2023;13:1129172. doi:10.3389/fcimb.2023.1129172
20. Rittig MG, Kaufmann A, Robins A, Shaw B, Sprenger H, Gemsa D, et al. Smooth and rough lipopolysaccharide phenotypes of Brucella induce different intracellular trafficking and cytokine/chemokine release in human monocytes. Journal of leukocyte biology. 2003;74(6):1045-55. doi:10.1189/jlb.0103015
21. Lapaque N, Moriyon I, Moreno E, Gorvel JP. Brucella lipopolysaccharide acts as a virulence factor. Current opinion in microbiology. 2005;8(1):60-6. doi:10.1016/j.mib.2004.12.003
22. Fernandez-Prada CM, Zelazowska EB, Nikolich M, Hadfield TL, Roop RM, 2nd, Robertson GL, et al. Interactions between Brucella melitensis and human phagocytes: bacterial surface O-Polysaccharide inhibits phagocytosis, bacterial killing, and subsequent host cell apoptosis. Infect Immun. 2003;71(4):2110-9. doi:10.1128/iai.71.4.2110-2119.2003
23. Gee JM, Valderas MW, Kovach ME, Grippe VK, Robertson GT, Ng WL, et al. The Brucella abortus Cu,Zn superoxide dismutase is required for optimal resistance to oxidative killing by murine macrophages and wild-type virulence in experimentally infected mice. Infect Immun. 2005;73(5):2873-80. doi:10.1128/iai.73.5.2873-2880.2005
24. Arellano-Reynoso B, Lapaque N, Salcedo S, Briones G, Ciocchini AE, Ugalde R, et al. Cyclic beta-1,2-glucan is a Brucella virulence factor required for intracellular survival. Nature immunology. 2005;6(6):618-25. doi:10.1038/ni1202
25. Khurana SK, Sehrawat A, Tiwari R, Prasad M, Gulati B, Shabbir MZ, et al. Bovine brucellosis - a comprehensive review. The veterinary quarterly. 2021;41(1):61-88. doi:10.1080/01652176.2020.1868616
26. Carvalho Neta AV, Mol JP, Xavier MN, Paixão TA, Lage AP, Santos RL. Pathogenesis of bovine brucellosis. Veterinary journal (London, England : 1997). 2010;184(2):146-55. doi:10.1016/j.tvjl.2009.04.010
27. Unuvar GK, Kilic AU, Doganay M. Current therapeutic strategy in osteoarticular brucellosis. Northern clinics of Istanbul. 2019;6(4):415-20. doi:10.14744/nci.2019.05658
28. Jin M, Fan Z, Gao R, Li X, Gao Z, Wang Z. Research progress on complications of Brucellosis. Frontiers in cellular and infection microbiology. 2023;13:1136674. doi:10.3389/fcimb.2023.1136674
29. Di Bonaventura G, Angeletti S, Ianni A, Petitti T, Gherardi G. Microbiological Laboratory Diagnosis of Human Brucellosis: An Overview. Pathogens (Basel, Switzerland). 2021;10(12). doi:10.3390/pathogens10121623
30. Bosilkovski M, Keramat F, Arapović J. The current therapeutical strategies in human brucellosis. Infection. 2021;49(5):823-32. doi:10.1007/s15010-021-01586-w
31. Hou H, Liu X, Peng Q. The advances in brucellosis vaccines. Vaccine. 2019;37(30):3981-8. doi:10.1016/j.vaccine.2019.05.084
32. Kornspan D, Brendebach H, Hofreuter D, Mathur S, Blum SE, Fleker M, et al. Protein Biomarker Identification for the Discrimination of Brucella melitensis Field Isolates From the Brucella melitensis Rev.1 Vaccine Strain by MALDI-TOF MS. Front Microbiol. 2021;12:712601. doi:10.3389/fmicb.2021.712601