Brucella Zoonosis: Treatment and Prevention Guide

34

Samar Wafa Kabeer[1], Rana Muhammad Shahbakht[1], Ahsan Anjum[1], Momna Mehmood[1], Aziz Ul-Rahman[1], Zahid Fareed[2], Hafiza Tuba Ashiq[1], Yousra Anwar[3], Junaid Ali Khan[1] and Muhammad Asif Raza[1]

ABSTRACT

David Bruce, identified Brucella melitensis in 1886, initially known as Malta Fever. Themistocles Zammit later revealed its zoonotic origin in goats. Brucella is a zoonotic disease transmitted from animals to humans. Brucella is a non-spore-forming gram-negative coccobacilli, lack capsules and virulence genes. Despite being non-motile, they possess genes for flagellum construction. The Brucella genus comprised of nine species shows host-specific genomic similarities, challenging understanding of survival mechanisms and intracellular growth. In animals, Brucella enters via mucous membranes or skin, bypassing immune defenses in organs, causing persistent infections, especially in the reproductive tract, leading to abortion. Infected animals shed the bacteria in fluids. In humans, Brucellosis enters through contaminated products or direct contact with animals, inducing systemic symptoms. Chronic cases may result in skeletal issues or rare neurological complications. Brucellosis, a geographically dynamic disease, in which cases are prevalent in Central Asia and escalating across the Middle East. Despite successful eradication in certain regions, brucellosis persists globally, impacting animal production and public health. Brucella strains exhibit zoonotic potential, with B. melitensis posing the highest risk. Effective eradication efforts have reduced human cases in certain countries. Human brucellosis presents diagnostic challenges, relying on laboratory tests due to varied clinical manifestations. Culture isolation remains the gold standard, while serological tests like the Brucella agglutination test and PCR-based methods are essential. In cattle, the Brucella ring test and blood tests are key for monitoring and eradication efforts. Swine brucellosis lacks a reliable serological test, but buffered plate Brucella antigen tests are practical. Ovine and caprine brucellosis screening relies on tests like the Rose Bengal plate agglutination, complement fixation, and indirect ELISA tests. Brucellosis treatment challenges arise from intracellular adaptation of the bacteria. Combining doxycycline and streptomycin (DS) is the most effective, although parenteral administration poses challenges. The rifampicin-doxycycline (DR) oral regimen is an alternative, but less potent, requiring individualized consideration, monitoring, and follow-up for optimal outcomes. In high-prevalence areas, controlling and eradicating brucellosis involves vaccination and the removal of infected animals. Key vaccines include B. abortus strains 19 and RB51 for cattle and B. melitensis strain Rev1 for goats and sheep. Despite vaccination, total Brucella eradication requires additional measures and sound husbandry practices due to vaccines providing partial protection, especially in regions with elevated infection rates.

Keywords: Brucellosis, Zoonotic disease, Epidemiology, Diagnosis, Treatment, Vaccination

CITATION

Kabeer SW, Shahbakht RM, Anjum A, Mehmood M, Fareed Z, Rahman A, Ashiq HT, Anwar Y, Khan JA and Raza MA, 2023. Brucella Zoonosis: Treatment and Prevention Guide. In: Altaf S, Khan A and Abbas RZ (eds), Zoonosis, Unique Scientific Publishers, Faisalabad, Pakistan, Vol 4: 443-454. https://doi.org/10.47278/book.zoon/2023.168

CHAPTER HISTORY Received: 06-May-2023 Revised: 09-June-2023 Accepted: 13-July-2023

[1]Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan, 66000, Pakistan
[2]Veterinary Research Institute, Lahore, Pakistan
[3]Department of Biotechnology, Virtual University of Pakistan
***Corresponding author:** samar.wafa@mnsuam.edu.pk

1. INTRODUCTION

David Bruce (1855-1931), a British army physician, identified "Micrococcus melitensis" from the spleen of a man who died of "Malta Fever" in 1886. This condition was prevalent, although it was sometimes mistaken with other ailments, particularly malaria. However, with crucial discoveries and advancements in the late nineteenth and early twentieth century, a substantial knowledge of this mysterious disease emerged much later. However, for about two decades after the isolation of M. melitensis, the real nature of Malta fever remained a mystery, with it being misdiagnosed as a vector-borne disease (Godfroid et al. 2005). It wasn't until 1905 that Themistocles Zammit, a Maltese physician, accidentally revealed the disease's zoonotic origin. He isolated B. melitensis from milk of goat, which was a pivotal finding. It was thought that goats are immune to infection since they showed no indications of sickness after being injected with Brucella cultures (Wyatt 2005). The startling findings that healthy goats may function as disease carriers was heralded as one of the most significant advances in epidemiology. Benhard Bang, a Danish veterinarian, discovered B. abortus, as the causal agent responsible for cow abortion, or Bang's disease, in 1897 (Khurana et al. 2021). Alice Evans, a distinguished American scientist known for her important study on harmful microorganism in milk and dairy related products, eventually affirmed the link between Malta fever and Bang's disease. Following these discovery, the genus was renamed Brucella in honor of David Bruce (Spink 1956). Evans' pioneering study on Brucella was essential in arguing for pasteurization procedures to protect against human brucellosis in the United States (Garcell et al. 2016). This discovery challenged prior conceptions of the disease's spread and demanded a reevaluation of management methods related with its incidence on land and in the water. Brucellosis is a zoonotic illness, which means it may spread from animals to people and vice versa.

Domestic animals, such as cattle, goat, sheep, pigs, and different wildlife species, play a role as key reservoirs for Brucella bacteria (Alton and Forsyth 1996). Humans generally get the infection by directly coming into contact with infected animals or their products, including unpasteurized milk and dairy products, or through exposure to contaminated animal tissues or fluids. In both animals and humans, the sickness appears differently. Brucella infection in animals can cause reproductive difficulties, such as abortions and decreased fertility, which can have serious economic ramifications for the cattle business (Gwida et al. 2010). One of the difficulties in controlling brucellosis is that it can persist in animal populations even in the absence of obvious clinical indications (Potter, 2013). Infected animals can become asymptomatic carriers, occasionally releasing the germs and creating a continual danger of transmission to humans and other vulnerable animals. Control strategies for brucellosis include immunization of animals, culling of sick animals, and stringent cleanliness techniques, particularly in the dairy sector (Dadar et al. 2021). Advances in diagnostic tools and molecular biology have increased our understanding of the variety and epidemiology of Brucella strains in recent years. Brucella species and strains have been found, each with variable degrees of virulence and host specificity (Christopher 2010). This understanding has aided in the creation of focused control measures for various settings and locations. Furthermore, research efforts have been aimed on producing effective vaccines for both animal

and human brucellosis. Vaccination of animals has shown encouraging benefits in lowering the occurrence of brucellosis in some areas (Dadar et al. 2021). Human vaccine development, on the other hand, remains a problem due to the disease's complexity and the need to balance safety and efficacy. Moreover, Brucellosis has been the subject of countless important discoveries throughout history, owing to its ancient beginnings. From David Bruce's early isolation of the causal agent through Themistocles Zammit's observation of its zoonotic character and Bang and Evans' identification of distinct Brucella species, each contribution has played an important part in developing our understanding of this complicated illness (Edwards and Jawad 2006; Wyatt 2016 ; Ghanbari et al. 2020).

2. CAUSATIVE AGENTS/ETIOLOGY

Brucella spp. are intriguing facultative intracellular gram-negative coccobacilli that are not spore-forming or capsulated (Alton and Forsyth 1996). Despite being categorized as non-motile, they have all of the genes required to build a functioning flagellum, with the exception of the chemotactic system. These adaptable bacteria are classified as Proteobacteria alpha-2, together with Ochrobactrum, Rhizobium, Rhodobacter, Agrobacterium, Bartonella, and Rickettsia (Fretin et al. 2005). The Brucella genus now has nine identified species, seven of which harm domestic animals: B. abortus, B. suis, B. canis, B. ovis, B. neotomae, B. microti and B. melitensis. Furthermore, two species, B. ceti and B. pinnipedialis, prey on marine animals (Liu 2015). The first three terrestrial species are known as classical Brucella, with seven biovars reported for B. abortus, three for B. melitensis, and five for B. suis. The other species have not yet been classified as biovars (Liu 2015). Surprisingly, Brucella strains are classified according to the host species they primarily infect. Because of advances in genomics, ten genomic sequences encompassing five Brucella species have been sequenced: B. abortus, B. melitensis, B. ovis, B. suis, and B. canis (Halling et al. 2005). Furthermore, around 25 additional Brucella strains/species are being sequenced. According to these genome investigations, Brucella members have surprisingly comparable genome sizes and gene compositions. Each species has two circular chromosomes and an average genome size of roughly 3.29 Mb. Chromosome I is roughly 2.11 Mb in length, whereas Chromosome II is around 1.18 Mb in length. All Brucella genomes have a G+C content of around 57.2% for Chromosome I and 57.3% for Chromosome II (Bohlin et al. 2010). Surprisingly, despite their mostly intracellular lifestyle, a study of 10 published Brucella genomes reveals similar aberrant areas in both chromosomes, suggesting the effect of horizontal gene transfer (Wattam et al. 2009). Brucella does not have any traditional virulence genes that encode capsules, plasmids, pili, or exotoxins. As a result, our understanding of the variables influencing their survival in the host and growth inside phagocytic cells is restricted in comparison to other bacterial pathogens (Głowacka et al. 2018). Furthermore, the complexities of Brucella's interactions with its host continue to provide considerable problems, necessitating continued study to uncover the underlying processes.

3. PATHOGENESIS

3.1. ANIMALS

Brucella primarily infiltrates the animal host through mucous membranes or skin abrasions, and it can also spread through the respiratory tract or contaminated feed and water. Within the host, it evades the immune system by residing in macrophages and dendritic cells. Upon initial penetration, localized infections arise in lymph nodes, spleen, and other organs. Bacteria proliferate and form granulomas, providing protection against immune responses and antimicrobial treatments. Systemic dissemination occurs through the circulation, leading to persistent granulomatous lesions in organs like the liver, reproductive organs, and mammary glands. The disease in animals is characterized by long-lasting

infection, with a particular affinity for the reproductive system, leading to abortion, stillbirth, or low birth weight. Infected females experience persistent endometritis, placentitis, and pregnancy loss as the bacteria colonize the placenta and uterine lining. Infected animals shed Brucella in bodily fluids like milk, urine, and reproductive secretions, serving as a source of infection for other vulnerable species and perpetuating the transmission cycle (Alton and Forsyth 1996 ; López-Santiago et al. 2019).

4. HUMANS

Brucellosis, typically contracted through the ingestion of contaminated animal products like unpasteurized milk, cheese, and meat, or direct contact with sick animals, can enter the body through mucous membranes or skin abrasions. Within the host, Brucella infiltrates monocytes and macrophages, establishing residence and multiplying. It induces granulomas in various organs, including the spleen, liver, and bone marrow. The infection spreads systemically through the circulation. Common non-specific symptoms include fever, fatigue, headache, joint discomfort, and sweating. In humans, Brucellosis may become chronic, leading to recurrent fever episodes and associated symptoms. The bacteria can persist in the body for months or even years, resulting in relapses and long-term complications. Brucella exhibits a predilection for the skeletal system, frequently causing osteoarticular issues like arthritis and spondylitis in humans. In rare cases, the bacteria can invade the central nervous system, leading to neurological symptoms such as meningitis or encephalitis (Alton and Forsyth 1996 ; de Figueiredo et al. 2015).

5. IMMUNOBIOLOGY

The stealthy nature of Brucella is primarily attributed to the unique nature of its smooth lipopolysaccharide (LPS) on the cell surface. The elongated fatty acid molecules on the lipid A portion of Brucella LPS reduce its toxicity and immunogenicity, making it a weak TLR4 agonist. This property allows Brucella to attack host cells with less activity. Moreover, the rough brucellae lacks the O-polysaccharide component of LPS, exhibit cytotoxicity to macrophage cells (Paul de Figueiredo et al. 2015). Although a comparative analysis of the lipid A from smooth and rough organisms have not been conducted. The lack of cytotoxic activity in rough LPS suggests that the O-polysaccharide is essential for the stealthy behavior of the organism (Stranahan and Arenas-Gamboa 2021).

In addition to the weak Brucella LPS agonist activity, the organism expresses novel immune regulatory factors that suppress the innate immune response. One such factor is the TIR-containing protein, TcpB/BtpA, which interacts with cytoplasmic MyD88 adaptor like/TIRAP. TcpB prevents MyD88 binding to TIRAP, accelerating its degradation and impairing TLR signaling, leading to reduced proinflammatory cytokine production. Another protein, BtpB, interferes with TLR signaling through MyD88, inhibiting dendritic cell maturation. The redundant factors of TcpB/BtpA and BtpB functions may explain the failure to identify these immunoregulatory genes through simple transposon screens (Jiao et al. 2021). Lack of expression of tcpB resulting in increased immune activation, resulting in reduced overall lifespan of microorganism. TcpB may act through protein kinase B to inhibit the NF-κB–mediated proinflammatory response and induce IL-10 production, ultimately contributing to the Brucella stealthy behavior (Smith et al. 2013).

Protection against Brucella has been studied in a variety of animal models, including mice, guinea pigs, ruminants, nonhuman primates, and humans. A T helper cell type 1 (Th1) response, including CD4+ and CD8+ T cells, is essential for protection. Antibodies to LPS, particularly the O-polysaccharide, may contribute to protection, but the role of the T helper cell type 2 (Th2) humoral immune response is unclear (Silva et al. 2011). Cytokines, such as IL-12, interferon-γ, tumor necrosis factor-α, IL-1, and IL-6, play important roles in mediating both innate and adaptive immune responses against Brucella. Reports

suggest that IL-1–dependent induction of colony-stimulating factor increases neutrophil and macrophage influx into the spleen, contributing to protection.

A variety of antigen of innate immune system, including complement, opsonins, phagocytes, innate lymphocytes, and cytokines, confer passive resistance to intracellular killing mechanisms. However, the importance of the type IV secretion system (T4SS) in the long-term Brucella infection is becoming clearer. Brucellae, like other intracellular pathogens, modifies the innate immune response to create recurrent adhesion and ensure long-term persistence (Paul de Figueiredo et al. 2015). The organism avoids the innate immune response by stealthy infiltration into host cells and controls protein secretion, cellular trafficking, and bacterial replication to alter the course of both innate and adaptive immune response.

The failure of immunization against Brucella infection is associated with a weak immune response, partly controlled by the attenuated innate immune response. As a stealth invader, Brucella enters host cells through TLR ligand interaction without apparent activation of the innate immune response (Pellegrini et al. 2022). Knockout mice deficient in either TLR2 or TLR4 do not significantly affect the ability to control the pathogen. However, cells deficient in MyD88 maintain a two-log increase in bacterial infection, indicating redundancy in host functions (Fang et al. 2010). By Evading the innate immune response of the host, Brucella can gain a foothold, while subsequent stimulation contributes to the spread of infection.

6. WORLDWIDE SPREAD AND ECONOMIC SIGNIFICANCE

Brucellosis, a disease with a continually fluctuating geographical distribution, is undergoing epidemiological changes as a result of a variety of variables including hygienic, economical, and political issues, as well as increased international travel (Khoshnood et al. 2022). Cases of human brucellosis have been observed, especially in Central Asia, and there is a significant surge in its spread across numerous Middle Eastern nations (Seleem et al 2010). Except in places where bovine brucellosis (B. abortus) has been successfully eliminated (no documented cases for at least five years), this illness is common (Godfroid et al. 2010). Certain countries have achieved brucellosis-free status, including Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden, and the United Kingdom, as well as Mediterranean European nations, northern and eastern African countries, Near Eastern countries, India, Central Asia, Mexico, and Central and South America. In contrast, these areas are currently dealing with brucellosis and have not yet eliminated the disease. (Khurana et al. 2021). Although B. melitensis has not been found in certain places, there are no convincing reports of its eradication from small ruminants elsewhere in the world (Blasco 1997). Human brucellosis, while being a nationally notifiable and reportable illness in the majority of nations, is severely underreported, with official figures reflecting only a fraction of the real incidence. As a result, the real worldwide burden of human brucellosis remains unknown, with estimates ranging from 0 to 160 cases per 100,000 people (Lai et al. 2021). The economic consequences of brucellosis are significant over the world, impacting both animal production (by lower milk, abortion, and delayed conception) and population health (via treatment expenses and productivity loss). Official estimates in Latin America, for example, show yearly losses of about $600 million due to bovine brucellosis (Angara et al. 2016). Though brucellosis eradication initiatives can be costly, they are thought to be cost-effective, with estimates indicating that every dollar put in eradication efforts save cost of treatment.

7. ZOONOSIS

Five of the nine identified Brucella species may infect people, with B. melitensis being the most virulent and invasive, followed in descending order of severity by B. suis, B. abortus, and B. canis. The zoonotic potential of marine brucellae (B. ceti) is well known (Liu 2015). Notably, in the United States B. melitensis,

B. suis, and B. abortus are categorized as possible bio-weapons due to their high infectivity, particularly through aerosolization (Khurana et al. 2021). Early signs of brucellosis are fever, joint pain and fatigue which make epidemic diagnosis difficult (Jiang et al. 2019). Infected animals directly transmit the disease to humans, or humans contract it by consuming their products, particularly unpasteurized milk and dairy products like cheese made from sheep and goat milk (Abdali et al. 2020). Specific occupational groups, such as veterinarians, agricultural laborers, meat-packing workers and ranchers are more vulnerable (Mobo et al. 2010). While B. suis and B. abortus infections mostly afflict workers, B. melitensis infections are more common in the general population (Alton and Forsyth 1996). Sheep or goat milk with B. melitensis is an important source of human brucellosis globally, causing multiple outbreaks; in certain regions, B. melitensis is responsible for 99% of human brucellosis cases (Rossetti et al. 2017). Human infections have decreased significantly as a result of brucellosis eradication efforts in animal reservoirs. For instance, in the United States, as a result of national bovine brucellosis eradication programme significant decline in human cases over time was reported. Denmark and France had comparable success in eradicating human brucellosis through eradication campaigns (Meyer 1956). Brucellosis typically affects people who come into direct contact with infected animals and consume milk and dairy products that are not pasteurize. Despite the fact that Brucella is extremely contagious when inhaled, inhaling the germs is not a common mechanism of infection (Głowacka et al. 2018). Certain occupational groups, however, such as laboratory and slaughterhouse workers, face severe risks in this respect. Brucella spp. account for up to 2 percent of all laboratory-related infections, making them the most prevalent pathogens found in laboratories (Madut et al. 2019). In 1999, 11.9% of clinical microbiology laboratory employees in Spain had laboratory-acquired brucellosis, according to a study (Bouza et al. 2005).

8. IMPACT OF BRUCELLOSIS ON HUMAN

Undulant fever, marked by temperature variations from 37.8°C in the morning to 40.8°C in the afternoon, as well as nocturnal sweats generating a unique odor, chills, and weakness, are the most prevalent signs of brucellosis. Anorexia, malaise, headache, sleeplessness, arthralgia, sexual dysfunction, constipation and anxiousness are other common symptoms. Furthermore, human brucellosis is recognized for its complications, which can damage numerous internal organs and cause a variety of symptoms depending on the site of infection. Encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis are some of the possible consequences (Dadar et al. 2021). Pregnant women infected with Brucella may have spontaneous abortions, which occur most frequently in the first and second trimesters of pregnancy (Bosilkovski et al. 2020). Brucella endocarditis is an uncommon but severe complication that accounts for at least 80% of brucellosis fatalities (Raju et al. 2013). Due to a lack of proper medication during the acute phase, brucellae might become localized in various tissues and organs, resulting in a difficult-to-treat subacute or chronic condition (Khan and Zahoor 2018). Brucellosis symptoms and signs can be confused with those of other diseases such as enteric fever, rheumatic fever malaria, thrombophlebitis, TB, fungal infections, autoimmune disorders, tumors and cholecystitis. However, with vaccine strains, the illness course is frequently shorter and less severe. Direct transmission of brucellosis from person to person is extremely rare. Breastfeeding women, on the other hand, may pass the virus to their newborns, and sexual transmission has also been recorded.

9. IMPACT OF BRUCELLOSIS ON ANIMALS

Abortion is the most prevalent clinical indication of Brucella infection in numerous livestock species, including cattle, sheep, goats, pigs, and camels (Garin-Bastuji et al. 1998). B. abortus is the most common strain responsible for infection in cattle. They can, however, become transiently infected with B. suis and,

more often, B. melitensis when they share pasture or facilities with diseased pigs, goats, and sheep (Gumaa et al. 2020). Because B. melitensis and B. suis may be spread through cow's milk, they can constitute a major public health risk. Symptoms are observed in pregnant animals, including abortion (weak calves or premature or full-term delivery of dead calves), during the second half of gestation, particularly in the third trimester, as well as placental retention and metritis (Bosilkovski et al. 2020). Infected cows may see a 20-30% decrease in milk output (Dadar et al. 2021). Brucellae reside in the supra-mammary lymph nodes and mammary glands of 80% of infected animals, secreting the infection into their milk constantly throughout their lives (Meador et al. 1989). Although most infected cows only have one abortion, the placenta can be highly contaminated during successive seemingly normal calvings. B. melitensis is the major causative agent of brucellosis in goats. Goats can become infected with B. abortus in places where B. melitensis is absent. Late abortion, stillbirths, reduced fertility, and low milk production are all symptoms of Brucellosis in goats. Sheep brucellosis is divided into two types: ram epididymitis and classical brucellosis. The non-zoonotic agent B. ovis causes ram epididymitis, whereas classical brucellosis is produced by B. melitensis and, like goat brucellosis, poses a substantial public health danger. Aside from miscarriage, pigs might suffer from lameness, hind limb paralysis, orchitis, spondylitis, and, on rare occasions, metritis or abscesses. Camels can become infected with B. melitensis and B. abortus if they graze among infected sheep, goats, and cattle. Infected camel milk is a major cause of infection, particularly in the Middle East, where its importance is sometimes overlooked. B. canis is the major etiologic agent of brucellosis in dogs (Khurana et al. 2021). There have been isolated occurrences of brucellosis in dogs caused by B. abortus, B. suis, and B. melitensis. Dogs infected with B. canis may develop reproductive issues such as miscarriages in the third trimester, conception failures or still births, as well as other issues such as ophthalmic, musculoskeletal, or dermatologic diseases (Santos et al. 2021). Although people are vulnerable to B. canis, the danger is smaller than with traditional brucella.

10. BRUCELLOSIS TESTING AND DETECTION METHODS

Human brucellosis has a complicated clinical picture, making a diagnosis based merely on symptoms problematic (Yagupsky et al. 2019). In endemic areas, every episode of fever with an unknown etiology is frequently considered to be brucellosis, emphasizing the significance of establishing the diagnosis by laboratory tests. It is crucial to make an accurate and timely diagnosis since delays or misdiagnosis can lead to treatment failures, relapses, chronic disease courses, localized complications, and even high case fatality rates. A correct diagnosis requires a complete case history, especially in non-endemic locations, to rule out travel-associated brucellosis or the ingestion of contaminated milk products imported from endemic regions (Yagupsky and Baron 2005). The isolation of Brucella from blood, bone marrow, lymph nodes, or cerebrospinal fluids is the gold standard for diagnosing brucellosis in people (Yagupsky et al. 2019). However, due to its sluggish development and limited sensitivity, culture cannot serve as a screening test. The sensitivity of Brucella isolation is influenced by individual laboratory procedures, the amount of pathogen in clinical samples, the stage of illness, the use of antibiotics prior to diagnosis, the culture methods used, and the specific Brucella strain involved (Yagupsky et al. 2019). The detection sensitivity varies greatly, ranging from 15 to 70 percent in individuals with acute infection and being considerably less in people with chronic illness (Yagupsky et al. 2019). The lysis centrifugation approach has recently demonstrated excellent results, with higher percentages of positive blood cultures (91% in acute brucellosis and 74% in chronic brucellosis) (Mantur and Mangalgi 2004). The existence of antibodies against the O-side chain of Brucella lipopolysaccharide is revealed by serological studies, which assess the serum's capacity to agglutinate a standardized quantity of dead B. abortus (Monreal et al. 2003). Brucella-specific IgM antibodies, followed by IgG antibodies, often develop in the last days of first week of the

disease and remain the most common and relevant markers for brucellosis diagnosis in the laboratory (Al Jindan et al. 2019). Furthermore, as compared to handling Brucella cultures, these agglutination tests are quicker and lower the risk of laboratory-acquired infections. It is important to note, however, that these serological assays are ineffective for identifying infections caused by B. canis, a strain that is inherently O-side chain lacking (Mol et al. 2020). The standard tube Brucella agglutination test is routinely used to diagnose acute brucellosis (Seleem et al. 2010). In chronic brucellosis, however, the 2-mercaptoethanol test and complement fixation tests are employed to detect current infection even when agglutination titers revert to low levels (Buchanan and Faber 1980). The 2ME test is identical to the standard tube Brucella agglutination test, except that leaving IgM antibodies inactive, 2ME is added to destroy disulfide bonds (Seleem et al. 2010). Other helpful diagnostic procedures for human brucellosis exist in addition to the aforementioned tests. The Rose Bengal test, counter immune-electrophoresis, Coombs test, immunocapture agglutination test, latex agglutination, and the indirect enzyme-linked immunosorbent assay are examples of these (Seleem et al. 2010). The use of polymerase chain reaction (PCR)-based tests for molecular diagnosis of human brucellosis has been recommended as a more helpful and sensitive method (Navarro et al. 2004). Such approaches, however, have not yet been completely verified for normal laboratory usage. Brucellosis testing in livestock is often done as part of monitoring efforts and disease eradication except for diagnostic purposes. Each country has its own policy regarding livestock testing. The Brucella ring test, which identifies antibodies in pooled milk samples from dairy herds, and the market cattle identification blood test, which analyzes serum antibodies in blood samples, are the two principal procedures for evaluating brucellosis in cattle in the United States (Godfroid et al. 2010). No serological test for swine brucellosis has been proved to be reliable for routine diagnosis. In contrast, buffered Brucella antigen tests, including the Rose Bengal plate agglutination test and the buffered plate agglutination test, are more accurate in practice in comparison with other tests for identifying infected herds (Lucero and Bolpe 1998). Rose Bengal plate agglutination, complement fixation, and indirect ELISA tests are commonly suggested for screening flocks and individual animals in the diagnosis of ovine and caprine brucellosis (Blasco et al. 1994). To summarize, human brucellosis is a difficult illness to identify clinically due to its wide range of clinical manifestations. Laboratory testing are required to confirm the diagnosis and distinguish it from other febrile diseases. While Brucella culture remains the gold standard, its sluggish development and poor sensitivity make it unsuitable as a screening test. Serological tests are often employed for diagnosis, such as the standard tube Brucella agglutination test for acute cases and the 2-mercaptoethanol and complement fixation tests for chronic infections. Molecular diagnostics employing PCR-based tests shows promise, but further research is needed. Testing is essential in cattle for disease eradication and monitoring programs, with different tests recommended depending on the species.

11. TREATMENT GUIDE

Treatment failures and relapses in brucellosis are prevalent due to Brucella's capacity to adapt inside its intracellular habitat, such as macrophages, and can be impacted by the medication combination utilized and patient compliance (Alavi and Alavi 2013 ; Mode et al. 2022). Because monotherapies with single antibiotics have been associated with significant rates of recurrence, the most successful way to treating brucellosis entails combining two medications. Due to its fewer side effects and lower recurrence rates, the use of streptomycin with doxycycline has emerged as the current effective treatment choice for instances of localized and acute brucellosis (Yousefi-Nooraie et al. 2012; Alavi and Alavi 2013). However, neither streptomycin nor doxycycline can effectively limit intracellular brucella proliferation. Despite its success, the DS regimen has disadvantages, most notably the requirement for streptomycin administration through parenteral route for three weeks, making it less practicable and less favored by patients. A

combination of parenteral gentamicin (5 mg/kg) and doxycycline therapy (6 weeks) for seven days has been deemed an appropriate replacement, since it provides reasonable effectiveness and enhanced convenience (Roushan et al. 2006). The World Health Organization (WHO) has long regarded DS combinations as the gold standard for brucellosis treatment (Alavi and Alavi 2013). However, in 1986, revision of recommendations by the Joint FAO/WHO Expert Committee on Brucellosis for treating adult acute brucellosis and introduced the use of rifampicin (600-900 mg/day orally) in combination with doxycycline (200 mg/day orally) as the regimen of choice, commonly known as the DR regimen (Falagas and Bliziotis 2006). Concerns regarding streptomycin's parenteral administration and the need for more accessible treatment choices motivated the decision to revise the guidelines. Nonetheless, studies comparing the efficacy of the DR regimen to the classic DS combination have found that the DS regimen is still more successful, particularly in acute brucellosis patients (Solera et al. 1995). The greater effectiveness of the DS combination may be attributable to streptomycin's powerful bactericidal action against Brucella, particularly in its acute form. While the DR regimen provides an oral option, it may not achieve the same level of bacterial eradication, increasing the likelihood of recurrence, particularly in acute patients. When deciding on the most effective treatment plan, it is critical to evaluate the individual circumstances of each patient as well as the strain of Brucella involved. Drug resistance trends, patient compliance, and the severity of the disease should all be considered. Close monitoring and follow-up assessments of patients during therapy are required to achieve effective results and limit the chance of recurrence. Because of Brucella's intracellular localization and capacity to adapt within host cells, brucellosis is a difficult infectious illness to cure. Combining two antibiotics, such as doxycycline and streptomycin (DS), has been shown to be the most effective treatment method, especially in localized and acute brucellosis. Among the treatment regimens available, the DS regimen stands as the gold standard, parenteral administration can be inconvenient and unpopular with patients. The DR regimen (rifampicin with doxycycline) gives an oral option as well, but it may be less effective, particularly in acute instances. Individual patient features and bacterial characteristics must be carefully considered when choosing the best treatment plan, with continuous monitoring and follow-up to guarantee effective results and limit the chance of recurrence.

12. VACCINATION AND IMMUNITY

Vaccination of vulnerable hosts and the eradication of diseased animals are critical techniques for controlling and eradicating this zoonosis in high-prevalence areas. B. abortus strain 19 and the USDA-approved strain RB51 are the most often used vaccinations for bovine brucellosis (CDC 1998 ; Stevens et al. 1997). Unlike strain 19, strain RB51 does not interfere with serological diagnosis. The persistence of antibodies while employing the B. abortus strain 19 vaccine is mostly determined by the age of the animals at the time of inoculation (Simpson et al. 2018 ; Seleem et al. 2010). Successful eradication programs must strictly limit the age at which strain 19 immunization is permitted, as testing and killing, in conjunction with vaccination, are critical components of such efforts. B. melitensis strain Rev1 is regarded the best vaccine for brucellosis control in goat and sheep, especially when delivered through conjunctival route in normal doses (Blasco 1997 ; Goodwin and Pascual 2016). However, the Rev1 vaccination is extremely virulent and can cause abortions when administered during pregnancy (Hensel et al. 2020). Furthermore, the immunity response after vaccination is same as reaction reported following acquired infection, limiting the effectiveness of control measures. Efforts have been undertaken to generate novel live attenuated rough B. melitensis vaccines without the O-side chain, which have yet to be tested in the field (Yang et al. 2013). It is critical to emphasize that total eradication of Brucella cannot be based exclusively on vaccination, because Brucella vaccines only provide partial protection, which may be undermined in the

face of increased infection rates (Seleem et al. 2010). As a result, a successful vaccination program must be accompanied with sound husbandry practices. Live vaccines, such as strains 19-BA and 104M of B. abortus are presently exclusively utilized in the Russia and China (Heidary et al. 2022). These vaccinations are intended to protect humans against brucellosis, however they are only available in certain areas. Brucella infections in animals have serious economic and public health consequences, especially in underdeveloped nations. Vaccination, in conjunction with control methods and sound husbandry techniques, is critical for disease control and eradication success. Vaccines for individual animal species are available, each with its own set of benefits and drawbacks. While vaccinations are important, their effectiveness must be supplemented by comprehensive control tactics in order to effectively combat brucellosis.

REFERENCES

Abdali F et al., 2020. Prevalence of Brucella species in unpasteurized dairy products consumed in Shiraz province using PCR assay. Molecular Biology Research Communications 9(3): 117.

Al Jindan R et al., 2019. Clinical Interpretation of Detection of IgM Anti-Antibody in the Absence of IgG and; a Diagnostic Challenge for Clinicians. Polish journal of microbiology 68(1): 51-57.

Alavi SM and Alavi L, 2013. Treatment of brucellosis: a systematic review of studies in recent twenty years. Caspian journal of internal medicine 4(2): 636.

Alton G and Forsyth J, 1996. Brucella. Medical microbiology. 4th ed. New York: Churchill Livingstone.

Angara T et al., 2016. Assessment of the economic losses due to bovine brucellosis in Khartoum State, Sudan. International Journal of Technical Research and Applications 4(2): 85-90.

Blasco J, 1997. A review of the use of B. melitensis Rev 1 vaccine in adult sheep and goats. Preventive veterinary medicine 31: 275-283.

Blasco J et al., 1994. Efficacy of different Rose Bengal and complement fixation antigens for the diagnosis of Brucella melitensis infection in sheep and goats. Veterinary record 134(16): 415-420.

Bohlin J et al., 2010. Genomic comparisons of Brucella spp. and closely related bacteria using base compositional and proteome based methods. BMC evolutionary biology 10(1): 1-16.

Bosilkovski M et al., 2020. Human brucellosis in pregnancy–An overview. Bosnian journal of basic medical sciences 20(4): 415.

Bosilkovski M et al., 2020. Brucellosis in pregnancy: case reports with different outcomes in an endemic region. Acta Clinica Croatica 59(2): 338.

Bouza E et al., 2005. Laboratory-acquired brucellosis: a Spanish national survey. Journal of Hospital Infection 61(1): 80-83.

Buchanan TM and Faber L, 1980. 2-mercaptoethanol Brucella agglutination test: usefulness for predicting recovery from brucellosis. Journal of clinical microbiology 11(6): 691-693.

CDC A, 1998. Human exposure to Brucella abortus strain RB51--Kansas, 1997. MMWR Morb Mortal Wkly Rep 47:172-175.

Christopher S, 2010. Brucellosis: review on the recent trends in pathogenicity and laboratory diagnosis. Journal of laboratory physicians 2(02): 055-060.

Dadar M et al., 2021. Isolation of Brucella melitensis biovar 1 from human milk confirms breastfeeding as a possible route for infant infection. Microbial Pathogenesis 157: 104958.

Dadar M et al., 2021. Importance of brucellosis control programs of livestock on the improvement of one health. Veterinary Quarterly 41(1): 137-151.

de Figueiredo P et al., 2015. Pathogenesis and immunobiology of brucellosis: review of Brucella–Host Interactions. The American journal of pathology 185(6): 1505-1517.

Edwards C and Jawad AS, 2006. History of brucellosis. Journal of the Royal Society of Medicine 99(2): 54-54.

Falagas ME and Bliziotis IA, 2006. Quinolones for treatment of human brucellosis: critical review of the evidence from microbiological and clinical studies. Antimicrobial agents and chemotherapy 50(1): 22-33.

Fang J et al., 2010. The role of TLR2, TLR3, TLR4, and TLR9 signaling in the pathogenesis of autoimmune disease in a retinal autoimmunity model. Investigative Ophthalmology and Visual Science 51(6): 3092-3099.
Fretin D et al., 2005. The sheathed flagellum of Brucella melitensis is involved in persistence in a murine model of infection. Cellular microbiology 7(5): 687-698.
Garcell HG et al., 2016. Outbreaks of brucellosis related to the consumption of unpasteurized camel milk. Journal of infection and public health 9(4): 523-527.
Garin-Bastuji B et al., 1998. Brucella melitensis infection in sheep: present and future. Veterinary research 29: 255-274.
Ghanbari MK et al., 2020. One health approach to tackle brucellosis: a systematic review. Tropical medicine and health 48: 1-10.
Głowacka P et al., 2018. Virulence Factors, Pathogenesis and Treatment. Polish journal of microbiology 67(2): 151-161.
Godfroid J et al., 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Veterinary research 36(3): 313-326.
Godfroid J et al., 2010. Diagnosis of brucellosis in livestock and wildlife. Croatian medical journal 51(4): 296-305.
Goodwin ZI and Pascual DW, 2016. Brucellosis vaccines for livestock. Veterinary immunology and immunopathology 181: 51-58.
Gumaa M et al., 2020. Specific detection and differentiation between Brucella melitensis and Brucella abortus by a duplex recombinase polymerase amplification assay. Frontiers in Veterinary Science 7: 539679.
Gwida M et al., 2010. Brucellosis–regionally emerging zoonotic disease? Croatian medical journal 51(4): 289-295.
Halling SM et al., 2005. Completion of the genome sequence of Brucella abortus and comparison to the highly similar genomes of Brucella melitensis and Brucella suis. Journal of Bacteriology 187(8): 2715-2726.
Heidary M et al., 2022. Evaluation of brucellosis vaccines: a comprehensive review. Frontiers in Veterinary Science 9: 925773.
Hensel ME et al., 2020. Vaccine candidate Brucella melitensis 16M vjbR is safe in a pregnant sheep model and confers protection. Msphere 5(3): 10.
Jiang W et al., 2019. Epidemiological characteristics, clinical manifestations and laboratory findings in 850 patients with brucellosis in Heilongjiang Province, China. BMC infectious diseases 19: 1-6.
Jiao H et al., 2021. The Mechanism of Facultative Intracellular Parasitism of Brucella. International Journal of Molecular Sciences 22(7): 3673.
Khan MZ and Zahoor M, 2018. An overview of brucellosis in cattle and humans, and its serological and molecular diagnosis in control strategies. Tropical medicine and infectious disease 3(2): 65.
Khoshnood S et al., 2022. Prevalence, diagnosis, and manifestations of brucellosis: A systematic review and meta-analysis. Frontiers in Veterinary Science 9: 976215.
Khurana SK et al., 2021. Bovine brucellosis–a comprehensive review. Veterinary Quarterly 41(1): 61-88.
Lai S et al., 2021. Human brucellosis: an ongoing global health challenge. China CDC Weekly, 3(6): 120.
Liu D, 2015. Brucella. Molecular Medical Microbiology 8: 1781-1788.
López-Santiago R et al., 2019. Immune response to mucosal brucella infection. Frontiers in immunology 10:1759.
Lucero NE and Bolpe JE, 1998. Buffered plate antigen test as a screening test for diagnosis of human brucellosis. Journal of clinical microbiology 36(5): 1425-1427.
Madut NA et al., 2019. Sero-prevalence of brucellosis among slaughterhouse workers in Bahr el Ghazal region, South Sudan. BMC infectious diseases 19(1): 1-7.
Mantur BG and Mangalgi SS, 2004. Evaluation of conventional castaneda and lysis centrifugation blood culture techniques for diagnosis of human brucellosis. Journal of clinical microbiology 42(9): 4327-4328.
Meador V et al., 1989. Pathogenesis of Brucella abortus infection of the mammary gland and supramammary lymph node of the goat. Veterinary pathology 26(5): 357-368.
Meyer K, 1956. Trends in brucellosis control. Public Health Reports 71(5): 511.
Mobo BHP et al., 2010. Occupational health of animal workers. Human-Animal Medicine 3: 343.
Mode S et al., 2022. Antibiotic persistence of intracellular Brucella abortus. PLoS neglected tropical diseases 16(7): 10635.

Mol JP et al., 2020. Diagnosis of canine brucellosis: comparison of various serologic tests and PCR. Journal of Veterinary Diagnostic Investigation 32(1): 77-86.

Monreal D et al., 2003. Characterization of Brucella abortus O-polysaccharide and core lipopolysaccharide mutants and demonstration that a complete core is required for rough vaccines to be efficient against Brucella abortus and Brucella ovis in the mouse model. Infection and immunity 71(6): 3261-3271.

Navarro E et al., 2004. Diagnosis of human brucellosis using PCR. Expert Review of Molecular Diagnostics 4(1): 115-123.

Pellegrini JM et al., 2022. Immunosuppressive Mechanisms in Brucellosis in Light of Chronic Bacterial Diseases. Microorganisms 10(7) 1260.

Potter ME 2013. Foodborne Infections and Intoxications: Chapter 15. Brucellosis. Elsevier Inc. Chapters.

Raju et al., 2013 Brucella endocarditis–A series of five case reports. indian heart journal 65: 72-77.

Rossetti et al., 2017. Caprine brucellosis: A historically neglected disease with significant impact on public health. PLoS neglected tropical diseases 11: e0005692.

Roushan et al., 2006. Efficacy of gentamicin plus doxycycline versus streptomycin plus doxycycline in the treatment of brucellosis in humans. Clinical infectious diseases 42: 1075-1080.

Santos et al., 2021. Canine brucellosis: an update. Frontiers in Veterinary Science 8: 5942

Seleem et al., 2010. Brucellosis: a re-emerging zoonosis. Veterinary microbiology 140: 392-398.

Silva TM et al., 2011. Laboratory Animal Models for Brucellosis Research. Journal of Biomedicine and Biotechnology 11: 518323.

Simpson et al., 2018 Immunological response to Brucella abortus strain 19 vaccination of cattle in a communal area in South Africa. Journal of the South African Veterinary Association 89: 1-7.

Smith JA et al., 2013. Brucella induces an unfolded protein response via TcpB that supports intracellular replication in macrophages. PLoS Pathogens 9(12): 1003785.

Solera et al.,1995. Doxycycline-rifampin versus doxycycline-streptomycin in treatment of human brucellosis due to Brucella melitensis. The GECMEI Group. Grupo de Estudio de Castilla-la Mancha de Enfermedades Infecciosas. Antimicrobial agents and chemotherapy 39: 2061-2067.

Spink, 1956. The nature of brucellosis. U of Minnesota Press.

Stevens et al., 1997. Brucella abortus strain RB51: a new brucellosis vaccine for cattle. Compendium 19: 766-775.

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: 713157.

Wattam et al., 2009. Analysis of ten Brucella genomes reveals evidence for horizontal gene transfer despite a preferred intracellular lifestyle. Journal of bacteriology 191: 3569-3579.

Wyatt, 2016. Lessons from the history of brucellosis.

Wyatt and H.V, 2005. How Themistocles Zammit found Malta Fever (brucellosis) to be transmitted by the milk of goats. Journal of the Royal Society of Medicine 98: 45

Yagupsky et al., 2005. Laboratory exposures to brucellae and implications for bioterrorism. Emerging infectious diseases, 11: 1180.

Yagupsky et al., 2019. Laboratory diagnosis of human brucellosis. Clinical microbiology reviews 33(1), 10.1128/cmr. 00073-00019.

Yang et al., 2013. Progress in Brucella vaccine development. Frontiers in biology 8: 60-77

Yousefi et al., 2012. Antibiotics for treating human brucellosis. Cochrane Database of Systematic Reviews 10.