Brucellar Arthritis

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History

Isolation of the *Brucella* sp. pathogen occurred in 1887 when the British physician Sir David Bruce managed to isolate the *Micrococcus melitensis* organism from the spleen of febrile patients dying in the island of Malta. He also described what we now know as brucellosis as a "long-term disease, with fever and profuse sweating, splenomegaly, frequent relapses, nerve or rheumatoid pain, inflammation of the joints, and orchitis" [1]. However, many centuries earlier Hippocrates in his book *Epidemics* already described a picture like brucellosis that was suffered by people living on the Mediterranean coast [2]. Brucellosis is also commonly known as Malta fever, Mediterranean fever, Cyprus fever, undulating fever, and Tifomalárica fever [3]. Kulowski and Vinke in 1932 described the first case of *Brucella* spondylitis after isolating *Brucella melitensis* from a paraspinal abscess [4]. On the other hand, in 1958 Ganado and Craig found that 2% of 6300 patients with brucellosis had spinal injuries. In the year 1951, in Argentina, de Anquin found an incidence of *Brucella* spondylitis in about 50% of their patients related to the variety *melitensis* [5, 6].

Epidemiology

Human brucellosis is endemic and is often recognized as an occupational disease in developing countries as well as in rural regions of developed countries [7]. Its worldwide incidence is often difficult to determine [8]. It represents a public health problem, especially the *melitensis* variety, for some Mediterranean countries, south-central Asia, and some regions of Africa and Latin America [9]. There is no gender predominance; however, women can develop a more severe form of brucellosis [10–12], with greater joint involvement [13] and more severe thrombocytopenia [14]. The pediatric population is less affected (they represent 20–25% of cases) [15]. It has been reported that the *melitensis* variety can produce symptomatology in 50% of the members of a family [10, 16].

The estimated incidence in the Mediterranean rim and in the Middle East is 100 cases per 100,000 people-years [17]. According to WHO, there are around five to six million cases of brucellosis worldwide and 500,000 new cases are reported annually [7, 18–20]. In the United States, 4–10% of the cases are recognized, perhaps by the influx of unpasteurized dairy products [21]. On the other hand, the incidence of *Brucella* spondylitis may range between 2%and 53% [22].

The Pathogen

Brucellosis constitutes a zoonosis in which the causative agent is *Brucella* sp., an intracellular bacterium transmitted from animals to humans [8]. This bacterium is a non-mobile gram-negative coccobacillus, of slow growth, aerobic, and catalase positive, belonging to the group A2 of *Alphaproteobacteria*, together with *Bartonella henselae* and *Agrobacterium tumefaciens* [23, 24]. There are several species of *Brucella*, which are classified according to the host that hosts them [25–29]:

- *B. melitensis* (the cause of Malta fever): Reservoirs are goats, sheep, and camels.
- *B. abortus* (cause of Bang's disease): Cause of abortion in cattle.
- *B. suis:* Cause of abortion in pigs.
- *B. canis*: Isolated in abortions of beagle dogs.
- *B. ovis* and *B. neotomae*: Isolated in sheep and wood rats, non-pathogens for humans.

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- *B. ceti*: Isolated from marine mammals such as whales, dolphins, and porpoises.
- *B. pinnipedialis*: Isolated in seals and walruses.
- *B. microti*: Isolated from red foxes in central Europe.

Of these, four are traditionally pathogenic for humans [7, 21, 30]: variety *melitensis*, *abortus*, *suis*, and *canis*.

Microorganisms can survive in unpasteurized goat cheese for more than 8 weeks and die within 60–90 days in cheese, resulting in lactic acid fermentation, and are eliminated in urine, feces, and animal-conception products and are viable for 40 days or more [8]. It is important to emphasize that the freezing of dairy products or meats does not guarantee the death of the bacteria unlike pasteurization and boiling [8]. Both the low number of virulent organisms and their adequate aerosolization capacity make it possible for this bacterium to be difficult to eradicate since its discovery, even in developed countries [2].

Transmission Mechanism

Humans happen to be accidental hosts. Infection is acquired through the gastrointestinal tract by means of the consumption of liver (viscera), raw meat, and milk products of goat, ovine, or bovine origin, especially if they are not pasteurized [15]. Transmission between humans is unlikely, but cases of transmission via the transplacental pathway [8], bone marrow transplantation [31], blood transfusion, and sexual intercourse are reported [11]. On the other hand, being considered an occupational disease among veterinarians, ranchers, and handlers of dairy products and meats, the most common transmission pathway is usually inhalation or conjunctival inoculation of the bacterium. Another route of common transmission between slaughterhouse workers is by contact of skin and mucous membranes eroded with bones and viscera of the animal [8].

Microbiology

The genome of *B. abortus* was decoded in 2001 and of the subtypes *melitensis* and *suis* in 2002 [32, 33]. For the detection of the bacterium, extended crops of up to 6 weeks are usually used using liquid or solid culture media or with the medium of Ruiz-Castañeda, since the crops are rarely positive before 10 days and could take up to several weeks. Automated cropping systems (such as the BACTEC) are more sensitive and usually positive within 7 days, but should be retained for 3 weeks. Bone marrow culture is seldom needed. The bacterium and its subtypes can also be detected using molecular diagnostic techniques such as the restriction fragment length polymorphism based on PCR (polymerase chain reaction) or the fluorescence in situ hybridization assay based on 16srRNA [8].

Pathogenesis

The bacterium, especially the subtype *melitensis*, is acquired by mouth. The incubation time is 2–3 weeks and includes invasion and multiplication within macrophages [15]. Its replication also usually takes place in dendritic cells, trophoblasts, microglia, fibroblasts, and epithelial and endothelial cells [2]. Immunity against *Brucella* infection is supported by the activation of antigen-specific T cells and in humoral responses. The pathogenicity of *Brucella* is very particular, since being an intracellular organism limits its exposure to the immune system; moreover, it does not present classical virulence factors and the lipopolysaccharides of its membrane are not typical. Currently, genes involved in structuring the virulence factors responsible for the processes of phagocytosis, fusion of phagolysosome, secretion of cytokines, and apoptosis have been characterized [34].

Upon invasion, *Brucella* adheres to the mucous membrane of epithelial cells through receptors containing sialic acid and sulfated residues [35], inducing activation of GTPases that are responsible for commanding the rearrangement of the cytoplasmic membrane to facilitate the entry of the bacterium, as well as activation of a mitogenic-dependent signaling pathway [36]. Once internalized, *Brucella* is detected by tissue lymphocytes and then transported by the lymphatic system to the regional lymph nodes and then via hematogenous spread to the rest of the organs, especially to the reticuloendothelial system. Localization in some organs can be associated with the presence of cellular infiltration with or without granulomatous formation, caseification, necrosis, or formation of abscesses. Shortly after its entry, both neutrophils and activated macrophages migrate to the initial point of entry. The innate immunity system is in charge of the initial response, which includes activation of Tγδ cells, natural killer (NK) cells, and CD4 and CD8 cells. The lipopolysaccharides (LPS) of the surface of the bacterium are recognized by these cells, which send signals to activate macrophages and facilitate phagocytosis of the bacterium. The bacterium enters macrophages by particular lipid-dependent structures of its own cytoplasmic membrane, known as uniform lipopolysaccharides (LPS-U), which are essential for its survival within infected macrophages. However, its immunogenicity is greatly inferior to the LPS of other gram-negative agents. It is believed that the unnoticed nature of *Brucella* is due in part to their LPS since these are weak agonists of the TLR4 so they would activate weakly the PI3K [37].

Tγδ cells promote the initial production of IFN-γ, TNF-α, and other cytokines, which become cytotoxic for monocytes

infected by *Brucella* and for the bacterium itself, hindering its intracellular survival. The Th lymphocytes secrete cytokines that activate mechanisms of intracellular death of macrophages infected with *Brucella*, known as "oxidative burst" or "oxygen-based death," which consist of the production of hydrolytic enzymes and activation of the peroxide-halide system. Only 10% phagocytized bacteria manage to survive, which will go to a period of adaptation within the phagocyte. These bacteria will be lodged inside a special vacuole, called "*Brucella* container vacuole" (BCV), which acts as a replicative compartment or brucellosome [38], where the mechanisms are activated to produce acidification and with the same promotion of survival of bacteria [8]. In parallel, *Brucella* will express a type IV secretion system (T4SS) which allows it to survive and multiply, being essential for its prolonged permanence [39].

During the infection, the surviving *Brucella* progressively recover all their functions, especially the reactivation of the transcription-translation, including those related to the genes of virulence [40–42]. Within the adaptation strategies, *Brucella* creates transcription mechanisms that favor the inhibition of apoptosis of infected monocytes, prevent the maturation of dendritic cells, reduce antigen presentation, and reduce the activation of virgin T cells [43]. In addition, *Brucella* can withstand death by oxidative burst using the hydrogen peroxide-halide system-myeloperoxidase [8].

Several studies indicate that an immune defect occurs during the invasive phase of infection. Although TH1 cells are responsible for commanding the response to *Brucella*, especially the CD4 and CD8 T cells [44], disease will occur due to a deteriorated response of Th1, defective T-cell proliferation, defective production of IFNγ, and poor quality of the cytotoxic activity of NK. However, studies in murine agents showed that the role of these cells was almost negligible [45].

On the other hand, IL2 produced by B cells and macrophages favors the response by TH1 and the induction of IFN-γ, whose activity is maximized by TNF-α produced by macrophages and NK. Induction of colony-stimulating factor dependent on IL1 increases the infiltration of macrophages and neutrophils into the spleen. The splenocytes come to express high levels of mRNA for IL2, IFNγ, and IL10 and low levels of mRNA for IL4 [46]. T4SS is the factor that produces a state of long-lasting infection to *Brucella*, making it clear that resistance mechanisms are not sufficient for the success of infection [44]. Studies in murine systems showed evidence that TLR2 or TLR4 deficiency generates a poor ability to control infection, unlike those MyD88-deficient cells which suffer a dramatic increase during brucellar infection [47].

Brucella can withstand the death mediated by lysosome and acidification by phagosome, continuing its multiplication in the endoplasmic reticulum of macrophages without affecting the integrity of the host cell. It evades the intracellular destruction by restricting the fusion of BCV to the lysosome, since it modifies the structure of this vacuole as well as of the endoplasmic reticulum, so that the BCV acquires autophagic function and positivity for the protein 1 associated to lysosomal membrane [48]. Subsequently, organisms are released by induced cell necrosis and lysis.

The virulence mechanisms of the bacterium will determine the survival or death of the infected macrophages. It is believed that one of the factors that impede the cellular uptake of the organism is the absence of the sensory-regulatory system BvrR/BvrS, because it originates important changes in the external bacterial membrane [46]. *Brucella* protects the infected cells from apoptosis in a mechanism that uses IFN-γ or TNF-α. In the initial stage of infection, *Brucella* increases the activation of the pathway AMPc/PKA which regulates a variety of mechanisms that favor *Brucella* infection by preventing the removal of host cells and favoring that macrophages become apoptosis-resistant [8].

Antibody-specific production as a response from the host to *Brucella* occurs immediately after infection. During the first week, IgM versus LPS appears in the serum. A week later, IgG and IgA appear and their peaks are reached during the fourth week. The appearance of anti-LPS antibodies has a limited role in defense against infection; however, they are important in diagnosing the disease.

Diagnostic Methods

Both serological and bacteriological methods may be used for the detection of *Brucella*; within the serological methods [49] currently available are Rose Bengal and 2-mercaptoethanol, molecular tests include PCR and ELISA, and bacteriological cultivation is also performed. In most cases diagnosis will be carried out by serology [50, 51]. However, the isolation of the bacterium in a culture medium (blood, tissue, or bone marrow) is the one that will provide definitive diagnosis.

Serological Methods

- *Rose Bengal*: Used as a screening for brucellosis. It is a rapid test that specifically detects IgG1-type antibodies against *Brucella* sp. It allows discrimination from cross-reactions or false positives. It has high sensitivity in acute brucellosis, close to 99%, although low specificity [52]. It is not useful in the follow-up of patients because it remains positive despite good evolution of the treatment.
- *Plate agglutinations*: Introduced by Wright, this test detects both IgG and IgM antibodies [53] which will attack the smooth lipopolysaccharides (LPS), so it can

give *cross-reactions with other bacteria* (*Salmonella group N*, *Vibrio cholerae*, *Escherichia coli O157*, *Yersinia enterocolítica*, *Francisella tularensis*, and *Stenotrophomonas maltophilia*, among others) [54]. It reacts quickly at the onset of an infection and may remain positive up to 2 years after successful treatment [55].

- *Tube agglutinations*: This test provides quantitative information by giving the result in titers in relation to the immune response against *Brucella* antigens. It is the most widely used technique in endemic countries [56]. Serial agglutination of serum in tube is carried out. It detects IgG2 and IgM antibodies. A titer ≥1:80 is considered positive in non-endemic regions and titer≥1:320 or even ≥1:160 in endemic regions. The main limitations that this test presents are that it takes a long time to do it, people in contact with livestock in endemic areas can show high degree of antibodies against *Brucella*, there is possibility of cross-reaction with other bacteria, and this test cannot identify acute cases from chronic [57–59].
- *2-Mercaptoethanol*: Used to detect IgG antibodies. It is based on the degradation of IgM due to the action of the radical thiol containing 2-mercaptoethanol. It is very useful in chronic infection in which the tube agglutination test may exhibit a low titer, since the serum will contain only IgG antibodies. In addition, decreased titers of IgG would indicate efficacy of treatment.
- *Coombs test*: It is not routinely performed due to its complexity, takes a long time to perform, is laborious, and needs a trained staff [56]. Nevertheless, it is useful in situations of a prozone phenomenon where false negatives can be obtained [60, 61] and where the evidence of agglutinations is negative despite having an evident clinical picture [62]. It is also the most sensitive method to confirm relapses [53].
- *ELISA*: This is a rapid test with a sensitivity and specificity greater than 80%. This test allows to measure the humoral immune response through the detection of IgM, IgG, and IgA antibodies [63–65] facilitating a better understanding of the condition of the disease. An advantage of its use is that it allows screenings of several patients to be performed simultaneously [66]. On the other hand, it has been reported to present high sensitivity to detect neurobrucellosis [53].

Regarding this technique, Mantur et al., to know its diagnostic certainty, published a study with 92 patients with clinical suspicion of brucellosis. All patients underwent tube agglutination for *Brucella*, 2-mercaptoethanol, culture, and ELISA [56]. It was found that the crop was positive in 33.6% and the agglutinations were positive in 25%, while the ELISA detected the disease in 60.9% of cases, reaching a sensitivity of 100% although a specificity of 71.3%. This study was used to show that ELISA is more sensitive than the agglutinations when detecting the disease in its acute and chronic phases. These results were like those previously found by Gad and Kambal and by Ariza et al. in their respective studies [67, 68].

Another interesting aspect of this study is that the ELISA could identify elevated values of IgM and IgG antibodies at any time of the disease. Other reports showed similar results to those of Mantur [67, 69, 70]. There is another simplified method of the ELISA called lateral flow assay (LFA), which can be used in both acute and chronic phases, is easy to interpret, and has a sensitivity and specificity greater than 90% [71].

Molecular Detection

Serological tests are sufficient for diagnosis; however, due to the possibility of cross-reaction or subsensitive reaction in samples from regions with low prevalence of *Brucella* infection, these tests might be proven to be unspecific [72]. Polymerase chain reaction or PCR has become quite relevant in the diagnosis of *Brucella*. This test is based on the detection of bacterial genetic material in biological samples (blood, cerebrospinal fluid, urine, post-mortem tissues) of both human and animal specimens and consists in conducting a specific amplification of bacterial DNA, when combining specific markers with DNA polymerase [73]. It is a fast and precise technique that contributes to an early diagnosis, especially during the acute phase of brucellosis, in addition to being useful during post-treatment follow-up and early relapse detection [74, 75]. It allows detection of more than 10 species of *Brucella* sp., and in low-income regions it will be used as an additional test in special cases of difficult diagnosis.

Currently there are several PCR techniques, such as real-time PCR, multiple PCR, and nested and semi-nested PCR, among others that are in development. However, all these tests do not yet have a standardized procedure that allows them to be used in a massive and equitable way between the various laboratories [76].

- *Conventional PCR*: It turns out to be more sensitive than microbiological methods both for the diagnosis of early detection and for relapses [77–79]; however, studies carried out by Baddour MM et al. and Navarro et al. showed that the efficiency of this technique depends on the specificity of the primers used [80, 81]. On the other hand, it has been seen that high concentrations of DNA from leukocytes and heme compounds can affect the results of PCR [82].
- *PCR in real time*: The advantage with respect to the conventional technique is that it turns out to be more economical and also allows the quantification of the nucleic

acids (number of copies of DNA, levels of expression of mRNA, and in other contexts, the viral load) [83–88]. It is a highly reproducible technique and of low cost and high speed and very sensitive and specific (90–100%). It is useful in initial diagnosis and to differentiate states of activity, inactivity, and seropositivity [76].

- *Multiple PCR*: It turns out to be useful because, in addition to minimizing expenses, it can recognize many pathogens at the same time [87]. It has high sensitivity and specificity, proving to be an alternative to crops. It also allows detection of *Mycobacterium tuberculosis* and *Brucella* sp. complex simultaneously. So it turns out to be a practical tool for the differential diagnosis of extrapulmonary tuberculosis and complicated brucellosis [88–90].

Bacteriological Method

The crop turns out to be the gold standard for the diagnosis of *Brucella*. The isolation of the bacteria in blood culture is possible in 40–70% of the cases (*B. melitensis* or *suis*), but with lower yield in the cases of *B. abortus*. The conventional method is the biphasic system of Ruiz-Castañeda [91], which is characterized by having a long incubation time of 6 weeks and sensitivity of 90% in the acute phase and 20% in the chronic phase [92, 93]. It can be optimized by using the method modified by Gotuzzo et al. who added sodium polyethylene sulfonate and cysteine. There is another culture method known as lysis centrifugation method [94] which differs from the previous one for the short time it takes to obtain the result [95]. Its sensitivity during the acute phase is also 90% and less than 70% in the chronic phase [94, 96]. Several publications indicate that the best method is culture of bone marrow versus repeated blood culture in two opportunities [97, 98] with a yield of 92% and with rapid growth. Culture of bone marrow is useful in situations that have high clinical suspicion against negative results of serological studies (recurrent uveitis, unexplained fever, hematologic abnormalities) [97, 99–101].

Clinical Spectrum

Brucellosis is an entity characterized by nocturnal fever, arthralgias, sweating, and splenomegaly. The most frequently affected organ systems are [102]:

- Osteoarticular in 20–30% of cases. It can be manifested by the presence of sacroiliitis, spondylitis, peripheral arthritis, osteomyelitis, or bursitis [9, 103].
- Genitourinary by orchiepididymitis with 40% of cases.
- Hepatic abscess at 1%.
- CNS involvement at 1–2%.
- Cardiovascular or endocarditis with less than 1%.

Clinical Presentation

Brucellosis can be acute, subacute or undulating, and chronic [15].

- *Acute*: Nocturnal fever greater than 38 °C, sweating, general malaise, weight loss, and arthralgias. One-third of patients develop arthritis, myalgia and back pain, anemia, leukopenia, and hepatic involvement in 40–50%.
- *Subacute or undulating*: It happens after 2 months. It is the most common form of presentation in endemic areas, becoming the cause of fever of unknown origin [104], persisting up to 1 year. Hepatic and articular compromise is common.
- *Chronic*: Lasts longer than 1 year. Two types of patterns are described:
  - In the first there is back pain, arthralgias, sweating, and depressive mood, like chronic fatigue syndrome.
  - The second pattern is characterized by involvement of a more localized area as it occurs in spondylitis or uveitis, in the absence of fever or systemic symptomatology [104].

Osteoarticular Manifestations

Constitutional and musculoskeletal or osteoarticular involvements are the most common clinical manifestations seen in human brucellosis. Majority of infected individuals, more than 70%, exhibit both fever and general malaise during the acute phase, while 10–60% exhibit arthralgias, back pain, peripheral arthritis, sacroiliitis, spondylitis, osteomyelitis, and bursitis (Table 6.1) [9, 15, 103, 105, 106]. However, it is necessary to emphasize that clinical presentation of these forms of articular involvement depends on the phase of the disease, since arthralgias and peripheral arthritis will be seen in more acute cases, while sacroiliitis will be seen in subacute cases and spondylitis in chronic phase [15].

Table 6.1 Osteoarticular manifestations

	Frequency	Brucellosis: clinical form
Peripheral arthritis Knee Hip Shoulders Sternum-clavicular joints	25–50%	Acute
Sacroiliitis	15–33%	Subacute
Spondylitis	5–12%	Chronic
Extraarticular manifestations Tendinitis Epicondylitis Bursitis Fibrositis	10–15%	–
Osteomyelitis	<1%	Subacute/chronic

On the other hand, the presence of tenosynovitis is usually not frequent, although it has rarely been described [9, 107].

Peripheral Arthritis

It is a common articular manifestation and may present as monoarticular or asymmetric oligoarticular presentation [106], becoming part of the differential diagnosis of seronegative spondyloarthritis. Joints commonly affected include the knee, hip, and shoulders; however there may also be involvement of sternum and sternum-clavicular joints [108]. It is usually seen in children and young adults [15]. Peripheral arthritis may be septic or reactive in origin. *Brucella* septic arthritis usually has a monoarticular presentation, with presence of the bacterium in the joint as a result of hematogenous spread, although it may also be due to an adjacent infection as would happen in osteomyelitis [8]. The bacterium may be isolated from the joint fluid provided that a suitable culture medium is used (although it does not occur in all cases) [15]; however, synovial biopsies are not useful for differentiating septic arthritis from reactive arthritis because they share the same histological characteristics [15]. Its prognosis is favorable if the appropriate antibiotic is chosen, requiring surgical cleaning of the joint only in cases of poor clinical evolution. On the other hand, in *Brucella*-induced reactive, clinical presentation is usually oligo- or polyarticular and the bacterium is not isolated from the joint [109]. Clinical improvement occurs with systemic anti-inflammatory therapy, although it can also spontaneously remit [110–113]. Polyarticular involvement, symmetric or asymmetric, with occasional presence of rheumatoid factor positivity, which might be transient, may also occur [106, 114]. Of interest, leukocyte values in peripheral blood are normal in both septic arthritis and spondylitis [8]. The study of synovial fluid reveals a count of leukocytes between 400 and 4000 cells/mm^3 with 60% polymorphonuclear, glucose may be reduced, and the culture could be positive in up to 50% of cases [8].

Sacroiliitis

Sacroiliac joint involvement is usually seen in children and young adults, being unilateral and with a more subacute presentation [15, 115, 116]. Gotuzzo et al. in their prospective study found that in their series of 163 cases with brucellosis, sacroiliitis was the second most common that affected joints, 33.1% [117]. However, this frequency can range from 9% to 57%, and unilateral involvement is seen in over 70% [118–120]. Laségue sign can frequently be found in patients with sacroiliac joint involvement [117]. Asymptomatic sacroiliitis with negative and/or normal Schober's test may be seen in 20–40% of patients [20]. HLA-B27 positivity may be present in 45% of patients and MRI is a more sensitive technique than plain x-ray in the diagnosis of sacroiliitis. Clinicians should have a high index of suspicion for the presence of asymptomatic sacroiliitis.

Spondylitis

Spondylitis has a global frequency between 2% and 53% [121], and it is seen in 5–10% of patients with brucellar arthritis [106, 117, 122]. It is clinically characterized by the triad: lumbar pain, nocturnal fever, and sweating [103]. Although it may occur in the subacute phase of the disease, it is mostly going to be present in the chronic phase, affecting people over 40 years [15]. It usually affects one or more lumbar vertebrae, having a greater predilection for L4 [8], following in frequency the thoracic vertebrae and lastly cervical. Patients often complain of lumbar pain exacerbated in decubitus position, a characteristic that makes it possible to differentiate it from non-inflammatory pathologies. Clinically, *Brucella* spondylitis is manifested by pain to deep percussion of the affected vertebrae with limitation of axial mobility; in cases of compression, the patient will refer dysesthesia in extremities, decreased muscle strength, and alteration of the osteotendon reflexes [103]. Infection begins with erosion at the edge of the antero-superior region of the vertebral body, which is the most vascularized area of the vertebra, then taking the appearance of a blunt or rounded edge [8, 106, 123–125]. The infection will compromise both the vertebral bodies and the intervertebral disk, and paravertebral abscesses rarely occur [8]. Diskitis or narrowing of the disk space constitutes the earliest sign of involvement, although the concomitant presence of blastic and lytic lesions and the rapid repair of lesions evidenced by the presence of sclerosis and osteophytes in "parrot beak," also characteristic presentations of *Brucella*, allow differentiation from spondylitis by tuberculosis or Pott's disease [126–129].

The Role of Imaging Techniques

The important role of imaging studies in the diagnosis of *Brucella* spondylitis has been clearly defined in the past several years. Imaging studies have been shown to be of great utility in the differential diagnosis of pyogenic or tubercular spondylitis, which constitutes their main differential diagnoses. Of all available techniques, it has been clearly demonstrated that magnetic resonance imaging (MRI) is the preferred imaging modality [130]. Imaging techniques, especially MRI, facilitate early diagnosis, especially in incipient phases when clinical suspicion is high.

Evidence of spinal involvement by imaging studies will depend on the phase or stage in which disease is diagnosed.

During acute phases involvement of multiple vertebrae and a variety of bony lesions can be seen [131, 132]. In early stages, osteolytic destruction is evidenced by the presence of lamellar bone dissolution of terminal plates and vertebral body and with low degree of bone destruction mediated by osteophytes [131, 132].

In chronic brucellar spondylitis, the center of vertebral bodies is involved by the inflammatory process and hardened, preventing the bone from being destroyed. This is evidenced by the presence of hyperplasia, sclerosis, and formation of osteophytes type "parrot beaks," eventually forming bony bridges [133]. Sclerosis will be expressed by the presence of hyperplasia of the vertebral body, proliferation of osteophytes, formation of bone bridges, sclerosis of the vertebral plaque, and osteogenesis of vertebrae [134].

Conventional Radiology: Spine X-Ray

Conventional x-ray fails to demonstrate structural spine changes in early stages of disease in the majority of patients [103]. However, bone destruction and proliferation were common in chronic stages, with vertebral bone hyperplasia, destruction, and sclerosis around the lesion [103]. Overall, lumbosacral spinal involvement is more common and seen in over 70% of patients, while cervical involvement is observed in less than 10%. Lateral osteophytes and disk space narrowing are also frequently seen, more than 70%, in chronic stages [103, 133].

Computed Axial Tomography (CAT)

As with conventional radiography, tomography does not add much in early stages of brucellar spondylitis, but it is highly informative in chronic stages. Both bone destruction and and sclerosis are observed in over 80% of patients [103]. Lamellar osteolytic destruction of the terminal plate and vertebral body, marginal osteophytes, and bony bridges are clearly identified by CAT in the majority of patients [133].

Magnetic Resonance Imaging (MRI)

MRI demonstrates vertebral involvement in over 90% of patients and intervertebral involvement in 80%. Areas of bone destruction will be shown to be hypodense in T1 sequences and hyperdense in T2 and STIR sequences (fat suppression), while peripheral sclerosis is associated with hypodensity in T1 and T2 and soft tissues issuing hypointensity in T1 and hyperintensity or isointensity in T2 [133]. Paravertebral abscesses can be present in about 8% of patients [117]. More recent studies have clearly confirmed the utility of MRI for the early diagnosis of brucellar spondylitis due to its high sensitivity in recognizing bone infection [103, 135, 136].

Extraarticular Involvement (Table 6.2)

- *Hematological*: Anemia, leukopenia with lymphocytosis, or thrombocytopenia may occur; the latter is so severe that in some cases, besides the administration of glucocorticoids, it may require splenectomy. Pancytopenia secondary to granulomas in the bone marrow may occur [137] and depending on the area may have an incidence between 2% and 14% in adult patients [138]. Although it is rare, mesenteric lymphadenitis as part of the acute phase of brucellosis may also occur [139].
- *Genitourinary*: Cases of orchiepididymitis are reported in endemic areas and can present an evolution so torpid that it may require orchiectomy [140]. It can be seen in adults and children and can be uni- or bilateral. Women may develop dysmenorrhea, tubo-ovarian abscesses, salpingitis, or cervicitis [8].
- *Neurological*: It is usually rare, but severe. It may be expressed by meningitis, encephalitis, or meningoencephalitis; it is reported in up to 5% of adults; in children it is a rare complication [125, 141–144]. Unlike tuberculosis, *Brucella* does not involve cranial pairs. The characteristics of CSF are like those of a bacterial meningoencephalitis; however *Brucella* is cultivable and can also be found in elevated agglutinations in CSF but is occasionally not usually detected [8].
- *Gastrointestinal*: Although it is rare, clinical hepatitis cases have been reported in 3–6% of adults, and it can be severe in concomitant cases of bone and hematologic involvement [29, 145–148].

Table 6.2 Constitutional and extraarticular manifestations

	Frequency
Fever	70–95%
Malaise	70%
Hematological (anemia, leukopenia, thrombocytopenia, or pancytopenia)	2–14%
Genitourinary (orchiepididymitis, tubo-ovarian abscesses, salpingitis, or cervicitis)	40%
Gastrointestinal (hepatitis)	3–6%
Neurological (meningitis, encephalitis, or meningoencephalitis)	<5%
Dermatological (erythema nodosum, purpura, and petechiae)	<5%
Cardiovascular (endocarditis, myocarditis, pericarditis, aortic abscesses)	<2%

- *Cardiovascular*: It is rare, but endocarditis, myocarditis, pericarditis, aortic abscesses, mycotic aneurysms, thrombophlebitis, and pulmonary embolism [8] may occur.
- *Dermatological*: Erythema nodosum, purpura, and petechiae may occur (although more as a result of thrombocytopenia), as well as chronic ulcers, cutaneous and subcutaneous abscesses, vasculitis, and superficial thrombophlebitis [8].

Brucellar Arthritis in Children

Brucella infection is uncommon in children, occupying this population group by 20–25% in all reported cases of human brucellosis [15]. Even studies carried out during between the 1950s and 1970s revealed that infection in pediatric patients was more frequent in school age, beginning to decrease its frequency in children under 7 years old [123, 124]. Children usually have acute and subacute forms of infection, developing a mild to moderate disease. Within the articular manifestations, which are also the most frequent during the development of the infection, peripheral arthritis tends to predominate [15]. In their series of cases of 84 children published in 1988, Gotuzzo et al. found that as in adults fever was the cardinal symptom in 93.8%, followed by anorexia in 73.5% and general malaise in 68.2%, while hepatomegaly was the main clinical finding with 77%, followed by adenomegaly at 61.1%; the presence of arthritis occupied a fifth place with 44% [117]. In addition, in the same study, it was found that the joint involvement was more frequent as the children reached older age and that this had preference for peripheral joint involvement in 69% followed by 23% by sacroiliac involvement; in addition the study drew attention to the lack of axial involvement.

More recent studies in pediatric populations have confirmed Gotuzzo et al.'s findings [139, 149–152].

Differential Diagnosis

Because fever is the predominant symptomatology, clinicians are obliged to rule out brucellosis in patients with fever of unknown origin or persistent fever despite antibiotic administration, within an appropriate clinical-epidemiological background. Diseases that may resemble brucellosis include typhoid fever, tuberculosis, infectious endocarditis, and acute rheumatic fever [153]. This diagnostic investigation should be carried out especially if fever occurs in the context of an immigrant patient who in addition to fever presents with arthralgias or peripheral arthritis, as evidenced in a series of cases recently published [154].

Treatment

Before opting for any therapy, it should be clear that the most appropriate treatment for brucellosis should reduce morbidity, prevent complications, and above all reduce the rate of relapse [155]. Another important aspect to consider is the surveillance of adverse events that may occur during treatment, to ensure proper adherence to it. The treatment, although basically its duration, depends on the phase in which the infection is detected as well as the type of organ that is compromised.

A characteristic feature of brucellosis is its capacity to relapse after completion of treatment, which usually occurs after 3 to 6 months or even after 2 years [156]. Relapse is due to its intracellular property that allows the organism to be protected from the mechanisms of defense of the host [156]. Therefore, it is necessary to opt for a drug with an adequate in vitro action, as well as intracellular action, being tetracyclines as the drug of choice and the cornerstone of therapy added to the synergistic action of rifampicin, even though in vitro studies have demonstrated resistance of *Brucella* to rifampicin when used as monotherapy [117, 157–162]. Other drugs that have shown great effect in lowering the rate of relapse by being associated with doxycycline are aminoglycosides, especially gentamicin [163], although there is greater evidence with streptomycin.

At the end of the 1980s, WHO proposed a standard treatment based on two dual therapies: doxycycline 200 mg/day for 6 weeks combined with rifampicin 600–900 mg/day for 6 weeks or with streptomycin 1 g/day for 2 to 3 weeks, either to be used as first-line treatment [164, 165]. Subsequent meta-analyses confirmed the superiority of the combination of doxycycline-streptomycin over doxycycline-rifampicin in terms of relapses and therapeutic failures [166–170]. The reason behind the low efficacy of treatment with doxycycline-rifampicin is that the concomitant administration of rifampicin causes decreased serum levels of doxycycline [171, 172]. One aspect that also began to be considered, in addition to clinical efficacy, is the possibility of provoking resistance in *Mycobacterium tuberculosis* with the prolonged use of rifampicin in endemic areas [173]. Despite this evidence, the reason why in some situations it is preferable to use doxycycline-rifampicin is due to the low cost of the medication as well as the ease of administration by mouth [174, 175] and the possibility that the aminoglycosides can provoke nephrotoxicity and ototoxicity when used for long periods. Treatment recommendations were made by WHO and the International Human Brucellosis Meeting (Table 6.3) [165, 176].

Quinolones are other drugs that according to the literature can also be used as part of the combined therapy either with doxycycline or rifampicin. Although it turns out to be an alternative, this combination has controversial results

Table 6.3 Treatment recommendations made by WHO and International Human Brucellosis Meeting

Treatment	Frequency	Route of administration	Duration
Doxycycline 100 mg Streptomycin 15 mg/kg	Twice daily Once daily	Orally Intramuscular	6 weeks 2–3 weeks
Doxycycline 100 mg Rifampicin 600–900 mg (one morning dose)	Twice daily Once daily	Orally	6 weeks
Doxycycline 100 mg Gentamicin 5 mg/kg	Once daily	Orally Endovenous or intramuscular	6 weeks 7 days
Above treaments + TMP-SMX 800/160 mg	Twice daily	Orally	6 weeks

Adapted from Ariza et al. [165]

Table 6.4 Treatment of brucellar arthritis in children

Treatment	Frequency	Route of administration	Duration
TMP/SMZ 8/40 mg/kg/d Streptomycin 30 mg/kg/d or Gentamicin 5 mg/kg/d	Twice Once	Orally Endovenous or intramuscular	6 weeks 3 weeks 7–10 days
TMP/SMZ 8/40 mg/kg/d Rifampicin 15 mg/kg/d	Twice Once	Orally	6 weeks
Rifampicin 15 mg/kg/d Streptomycin 30 mg/kg/d or Gentamicin 5 mg/kg/d	Once	Orally Endovenous or intramuscular	6 weeks 3 weeks 7–10 days

since several studies have found that there is not much difference between those groups that used quinolones (including ofloxacin and ciprofloxacin) and those who did not use quinolones [177–181]. A meta-analysis of randomized clinical studies published in 2008 concluded that the combination of a quinolone with rifampicin was less effective than treatment with doxycycline-rifampicin or doxycycline-streptomycin [182]. On the other hand, a study published in 2012, which compared the use of doxycycline-streptomycin versus doxycycline-rifampicin versus rifampicin-ofloxacin, found that the group that received doxycycline-streptomycin presented greater clinic response, lower relapse rate, and therapeutic failure rate [178, 183, 184]. However, a recent study published in 2016, which compared patients receiving dual therapy with doxycycline-rifampicin versus triple therapy with doxycycline-rifampicin-levofloxacin for 6 weeks, found that the relapse rate was higher in the first group (22.6% versus 9.3%), a result that was similar to those previously found by Akova et al., Karabay et al., and Solera et al. [170, 177, 178], showing that there is an increase in resistance to dual therapy in the last several years [185].

Dual therapy with doxycycline-streptomycin is the choice for osteoarticular involvement [170, 186], and Gotuzzo et al. suggested that any of the two first-line regimens for a period of 4 weeks, with streptomycin being administered IM for 2 weeks, should be appropriate [117]. In cases of spondylitis and osteomyelitis, the recommendation is to prolong therapy that could last several months, with doxycycline to be used for 8 or more weeks. Need for surgery occurs rarely [176]. *Brucella* sacroiliitis does not require specific treatment.

In the case of chronic brucellosis, since it is difficult to diagnose, Gotuzzo et al. have suggested the use of immunomodulators. Although there are different treatment alternatives, cases of recurrence continue to persist over the years; that is why studies with the use of immunomodulators are under development. This is the case of hydroxychloroquine, widely used in the management of joint involvement of connective tissue diseases and which apparently has a positive impact on brucellar infection by favoring the creation of an alkaline environment that counteracts the intracellular acidification produced by *Brucella*, managing to destroy the phagolysosome [187]. In a recent study that compared the use of doxycycline-streptomycin versus doxycycline-streptomycin-hydroxychloroquine, favorable results in terms of clinical response and relapses were found in the second group compared to the first [188]. More studies are still in development.

In the case of pediatric patients under 8 years of age, WHO recommends avoiding the use of all tetracyclines, including doxycycline. Although to date there is no therapy of choice, what is recommended to use in this type of population are the aminoglycosides, cotrimoxazole, or rifampicin in combination therapies. As in adults, monotherapy is avoided due to the frequency of relapses. Treatment is successful with the use of TMP-SMZ (8/40 mg/kg/day) for 6 weeks together with streptomycin 30 mg/kg/day intramuscular daily for 3 weeks or gentamicin 5 mg/kg/day intravenous or intramuscular for 7–10 days. Other alternative treatments are those shown in Table 6.4[176].

Conflicts of Interest *EMM*: None. *KVV*: None.

References

1. Bruce D. Note on discovery of a micrococcus in Malta fever. Practitioner. 1887;39:161–2.
2. De Figuereido P, Ficht TA, Rice-Ficht A, Rosetti CA, Adams G. Pathogenesis and immunobiology of brucellosis. Review of Brucella-Host interactions. Am J Pathol. 2015;185:1505–17.

3. Young EJ. Brucella spp. principles and practice of clinical bacteriology. 2nd ed. West Sussex: John Wiley&SonsLtd; 2006. p. 265–72.
4. Kulowski J, Vinke T. Undulant (Malta) fever spondylitis: report of a case, due to Brucella melitensis, bovine variety, surgically treated. JAMA. 1932;99:1656–9.
5. De Anquin C. Espondilitis bruselósica. II Jornadas Argentinas de Ortopedia. 1951:176–83.
6. Mandell GL, Bennett JE, Dolin R. Principles and practices of infectious disease, vol. 2. 5th ed. Filadelfia: Churchill Livingstone; 2000. p. 2386–93.
7. Pappas G, Papadimitriou P, Akritidis N, et al. The new global map of human brucellosis. Lancet Infect Dis. 2006;6:91–9.
8. Beeching NJ, Madkour MM. Chapter 28: brucellosis. In: Farrar J, editor. Manson's tropical diseases. 23rd ed. United Kingdom: Elsevier; 2014.
9. Elzein FE, Sherbeeni N. Brucella septic arthritis: case reports and review of the literature. Case Rep Infect Dis. 2016;2016:4687840.
10. Gotuzzo E, Carrillo C, Seas C, et al. Características epidemiológicas y clínicas de la brucelosis en 39 grupos familiares. RevEspInternInfectMicrobiolClin. 1989;7:519.
11. Goossens H, Marcelis L, Dekeyser P, et al. Brucella melitensis: person-to-person transmission? Lancet. 1983;1:773.
12. Gotuzzo E, Carrillo C, Seas C, et al. Caracteristicasepidemiólogicas y clinicas de la brucelosis en 50 grupos familiares. RevMedHered. 1990;1:8.
13. Gotuzzo E, Seas C, Guerra J, et al. Brucellar arthritis – study of 39 Peruvian families. Ann Rheum Dis. 1987;46:506.
14. Ulloa V, Rojas J, Gotuzzo E. Púrpura trombocitopénica asociada a brucelosis. Rev Med Hered. 1992;3:87.
15. Gotuzzo E, Pappas G. Chapter 40.Brucellosis. In: Guerrant RL, editor. Tropical infectious diseases. 3rd ed. Spain: Saunders- Elsevier; 2011.
16. Mousa ARM, Elhag KM, Hkogall M, et al. The nature of human brucellosis in Kuwait, study of 379 cases. J Infect Dis. 1988;10:211.
17. Al-Tawfiq JA, AbuKhamsin A. A 24-year study of the epidemiology of human brucellosis in a health-care system in Eastern Saudi Arabia. J Infect Public Health. 2009;2(2):81–5.
18. Wang D, Zhang S. Advances in treatment of acute brucellosis. ZhongGuoGan Ran Kong ZhiZaZhi. 2001;16:94–5.(In Chinese).
19. Shang DQ. Research advances in brucellosis disease. ZhongGuoGan Ran Kong ZhiZaZhi. 2004;19:204–12.(In Chinese).
20. World Health Organization in collaboration with Food and Agriculture Organization and World Organization for Animal Health. Brucellosis in humans and animals. Geneva: WHO; 2006.
21. Pappas G. The changing. Int J Antimicrob Agents. 2010;36:S8–S11.
22. Momjian R, George M. Atypical imaging features of tuberculous spondylitis: case report with literature review. J Radiol Case Rep. 2014;8(11):1–14.
23. Moreno E, Stackebrandt E, Dorsch M, et al. Brucella abortus 16S rRNA and lipid A reveal a phylogenetic relationship with members of the alpha-2 subdivision of the class Proteobacteria. J Bacteriol. 1990;172:3569.
24. DeLay J, Mannheim W, Segers P, et al. Ribosomal ribonucleic acid cistron similarities and taxonomic neighborhood of Brucella and CDC group Vd. Int J SystBacteriol. 1987;37:35.
25. Verger JM, Grimont F, Grimont PAD, et al. Brucella, a monospecific genus as shown by deoxyribonucleic acid hybridization. Int J SystBacteriol. 1985;35:292–5.
26. Corbel MJ. International Committee on Systemic Bacteriology Subcommittee on the Taxonomy of Brucella. Int J SystBacteriol. 1988;38:450.
27. Foster G, Osterman BS, Godfroid J, et al. Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. Int JSystEvolMicrobiol. 2007;57:2688.
28. Sohn AH, Probert WS, Glaser CA, et al. Human neurobrucellosis with intracerebral granuloma caused by a marine mammal. Emerg Infect Dis. 2003;9:485.
29. Scholz HC, Hubalek Z, Sedlacek I, et al. Brucella microti sp. nov., isolated from the common vole Microtus arvalis. Int J SystEvolMicrobiol. 2008;58:375.
30. Madkour MM. Brucellosis: overview. In: Madkour MM, editor. Madkour's brucellosis. 2nd ed. Berlin: Springer; 2001. p. 1–14.
31. Naparsteck E, Block CS, Slavin S. Transmission of brucellosis in bone marrow transplantation. Lancet. 1982;1:574.
32. Al Dahouk S, Tomaso H, Prenger-Berninghoff E, et al. Identification of Brucella species and biotypes using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Crit Rev Microbiol. 2005;31:191–6.
33. Al Dahouk S, Nöckler K. Implications of laboratory diagnosis on brucellosis therapy. Expert Rev Anti-Infect Ther. 2011;9:833–45.
34. Von Bargen K, Gorvel JP, Salcedo SP. Internal affairs: investigating the. FEMS Microbiol Rev. 2012;36:533–62.
35. Castañeda-Roldán EI, Avelino-Flores F, Dall'Agnol M, Freer E, Cedillo L, Dornand J, Girón JA. Adherence of Brucella to human epithelial cells and macrophages is mediated by sialic acid residues. Cell Microbiol. 2004;6:435–45.
36. Rossetti CA, Drake KL, Adams LG. Transcriptome analysis of HeLa cells response to Brucella melitensis infection: a molecular approach to understand the role of the mucosal epithelium in the onset of the Brucella pathogenesis. Microbes Infect. 2012;14:756–67.
37. Qin QM, Pei J, Ancona V, Shaw BD, Ficht TA, de Figueiredo P. RNAi screen of endoplasmic reticulum-associated host factors reveals a role for IRE1alpha in supporting Brucella replication. PLoSPathog. 2008;4:e1000110.
38. Kohler S, Foulongne V, Ouahrani-Bettache S, Bourg G, Teyssier J, Ramuz M, Liautard JP. The analysis of the intramacrophagicvirulome of Brucella suis deciphers the environment encountered by the pathogen inside the macrophage host cell. Proc Natl AcadSci U S A. 2002;99:15711–6.
39. Roux CM, Rolan HG, Santos RL, Beremand PD, Thomas TL, Adams LG, Tsolis RM. Brucella requires a functional Type IV secretion system to elicit innate immune responses in mice. Cell Microbiol. 2007;9:1851–69.
40. Lamontagne J, Forest A, Marazzo E, Denis F, Butler H, Michaud JF, Boucher L, Pedro I, Villeneuve A, Sitnikov D, Trudel K, Nassif N, Boudjelti D, Tomaki F, Chaves-Olarte E, Guzman-Verri C, Brunet S, Cote-Martin A, Hunter J, Moreno E, Paramithiotis E. Intracellular adaptation of Brucella abortus. J Proteome Res. 2009;8:1594–609.
41. Rambow-Larsen AA, Rajashekara G, Petersoen E, Splitter G. Putative quorum-sensing regulator BlxR of Brucella melitensis regulates virulence factors including the Type IV secretion system and flagella. J Bacteriol. 2008;190:3274–82.
42. Weeks JN, Galindo CL, Drake KL, Adams GL, Garner HR, Ficht TA. Brucella melitensisVjbR and C12-HSL regulons: contributions of the N-dodecanoylhomoserine lactone signaling molecule and LuxR homologue VjbR to gene expression. BMC Microbiol. 2010;10:167.
43. Billard E, Dornand J, Gross A. Brucella suis prevents human dendritic cell maturation and antigen presentation through regulation of tumor necrosis factor alpha secretion. Infect Immun. 2007;75:4980–9.
44. Young EJ, HasanjaniRoushan MR, Shafae S, Genta RM, Taylor SL. Liver histology of acute brucellosis caused by Brucella melitensis. Hum Pathol. 2014;45:2023–8.
45. Fernandes DM, Benson R, Baldwin CL. Lack of a role for natural killer cells in early control of Brucella abortus 2308 infections in mice. Infect Immun. 1995;63:4029–33.
46. De Figueiredo P, Ficht TA, Rice-Ficht A, Rossetti CA, Adams LG. Pathogenesis and immunobiology of brucellosis: review of Brucella–Host interactions. Am J Pathol. 2015;185(6):1505–17.
47. Salcedo SP, Marchesini MI, Lelouard H, Fugier E, Jolly G, Balor S, Muller A, Lapaque N, Demaria O, Alexopoulou L, Comerci DJ, Ugalde RA, Pierre P, Gorvel JP. Brucella control of dendritic cell

maturation is dependent on the TIR-containing protein Btp1. PLoS Pathog. 2008;4:e21.
48. Smith JA, Khan M, Magnani DD, Harms JS, Durward M, Radhakrishnan GK, Liu YP, Splitter GA. Brucella induces an unfolded protein response via TcpB that supports intracellular replication in macrophages. PLoS Pathog. 2013;9:e1003785.
49. Ministerio de Salud del Perú. Norma técnica de diagnóstico y tratamiento de brucelosis humana: N.T. No. 002-MINSA/DGSP-V.01/ Ministerio de Salud. Dirección General de Salud de las Personas. Dirección Ejecutiva de Atención Integral de Salud – Lima: Ministerio de Salud;2005. 23 p.
50. Ruiz-Castañeda M, Tovar R, Velez R. Studies on brucellosis in Mexico. Comparative study of various diagnostic tests and classification of the isolate bacteria. J Infect Dis. 1942;70:97.
51. Williams E. Brucellosis. Practitioner. 1982;226:1507.
52. Ruiz-Mesa JD, Sanchez-Gonzalez J, Reguera JM, Martin L, Lopez-Palmero S, Colmenero JD. Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. ClinMicrobiol Infect. 2005;11(3):221–5.
53. Christopher S, Umapathy BL, Ravikumar KL. Brucellosis: review on the recent trends in pathogenicity and laboratory diagnosis. J Lab Physicians. 2010;2(2):55–60.
54. Perry MB, Bundle DR. Lipopolysaccharide antigens and carbohydrates of Brucella. In: Adams LG, editor. Advances in brucellosis research. Austin: Texas A and M University; 1990. p. 76–88.
55. Almuneef M, Memish ZA. Persistence of Brucella antibodies after successful treatment of acute brucellosis in an area of endemicity. JClinMicrobiol. 2002;40(6):2313.
56. Mantur B, Parande A, Amarnath S, et al. ELISA versus conventional methods of diagnosing endemic brucellosis. Am J Trop Med Hyg. 2010;83(2):314–8.
57. Zourbas J, Masse L, Roussey A, David C, Maurin J, Torte J. Sampling survey on brucellosis among farmers and their families in IIIe-et-Vilaine (Brittany). Int J Epidemiol. 1977;6:335–43.
58. Gilbert GL, Beaton CP, Forsyth JR, Bell CO. An epidemiological survey of human brucellosis in three Victorian abattoirs. Med J Aust. 1980;1:482–6.
59. Daz R, Maravi-Poma E, Rivero A. Comparison of counterimmunoelectrophoresis with other serological tests in the diagnosis of human brucellosis. Bull World Health Organ. 1976;53:417–24.
60. Monir MM, Dennis LK. Brucellosis. In: Braunwald F, Kasper H, Lango J, editors. Harrison's principles of internal medicine. 15th ed. New York: McGraw-Hill Education; 2001. p. 986–90.
61. Serra J, Vinas M. Laboratory diagnosis of brucellosis in a ruralendemic area in northeastern Spain. Int Microbiol. 2004;7(1):53–8.
62. Young EJ. An overview of human brucellosis. Clin Infect Dis. 1995;21(2):283–9.
63. Araj GF, Kaufmann AF. Determination by enzyme-linked immunosorbent assay of immunoglobulin G (IgG), IgM, and IgA to Brucella melitensis major outer membrane proteins and whole cell heat-killed antigens in sera of patients with brucellosis. J Clin Microbiol. 1989;27(8):1909–12.
64. Osoba AO, Balkhy H, Memish Z, Khan MY, Al-Thagafi A, Al Shareef B, et al. Diagnostic value of Brucella ELISA IgG and IgM in bacteremic and non-bacteremic patients with brucellosis. J Chemother. 2001;13(Suppl 1):54–9.
65. Kostoula A, Bobogianni H, Virioni G, Tabatabai LB. Detection of Brucella IgG, IgM and IgA antibodies with ELISA method in patients with brucellosis. Clin Microbiol Infect. 2001;7(1):108.
66. Agasthya AS, Isloor S, Krishnamsetty P. Seroprevalence study of human brucellosis by conventional tests and indigenous indirect enzyme-linked immunosorbent assay. Sci World J. 2012;2012:104239.
67. Gad El-Rab MO, Kambal AM. Evaluation of a Brucella enzyme immunoassay test (ELISA) in comparison with bacteriological culture and agglutination. J Infect. 1998;36:197–201.
68. Ariza J, Pellicer T, Pallares R, Foz A, Gudiol F. Specific antibody profile in human brucellosis. ClinInfectDis. 1992;14:131–40.
69. Gazapo E, Gonzalez Lahoz J, Subiza JL, Baquero M, Gil J, de la Concha EG. Changes in IgM and IgG antibody concentrations in brucellosis over time: importance for diagnosis and follow-up. J Infect Dis. 1989;159:219–25.
70. Reddin JL, Anderson RK, Jenness R, Spink WW. Significance of 7S and macroglobulin Brucella agglutinins in human brucellosis. N Engl J Med. 1965;272:1263–8.
71. Roushan MR, Amin MJ, Abdoel TH, Smits HL. Application of a user-friendly Brucella-specific IgM and IgG antibody assay for the rapid confirmation of Rose Bengal-positive patients in a hospital in Iran. Trans R Soc Trop Med Hyg. 2005;99(10):744–50.
72. Pabuccuoglu O, Ecemis T, El S, Coskun A, Akcali S, Sanlidag T. Evaluation of serological tests for diagnosis of brucellosis. Jpn J Infect Dis. 2011;64(4):272–6.
73. Ministerio de Salud del Perú. Norma técnica de salud para la atención integral de la persona afectada con brucelosis. NTS N° -MINSA/DGSP-V.01 Ministerio de Salud.Dirección General de Salud de las Personas. Dirección Ejecutiva de Atención Integral de Salud – Lima: Ministerio de Salud;2009.
74. Mitka S, Anetakis C, Souliou E, Diza E, Kansouzidou A. Evaluation of different PCR assays for early detection of acute and relapsing brucellosis in humans in comparison with conventional methods. J ClinMicrobiol. 2007;45(4):1211–8.
75. Al Ajlan HH, Ibrahim AS, Al Salamah AA. Comparison of different PCR methods for detection of Brucella spp. in human blood samples. Pol J Microbiol. 2011;60(1):27–33.
76. Wang, et al. Polymerase chain reaction–based assays for the diagnosis of human brucellosis. Ann Clin Microbiol Antimicrob. 2014;13:31.
77. Morata P, Queipo-Ortuño MI, Reguera JM, García-Ordoñez MA, Pichardo C, Colmenero JD. Posttreatment follow-up of brucellosis by PCR assay. J ClinMicrobiol. 1999;37(12):4163–6.
78. Elfaki MG, Al-Hokail AA, Nakeeb SM, Al-Rabiah FA. Evaluation of culture, tube agglutination, and PCR methods for the diagnosis of brucellosis in humans. Med SciMonit. 2005;11(11):MT69–74.
79. Briones Lara E, del C Palacios Saucedo G, Martínez Vázquez IO, Morales Loredo A, del PilarBilbao Chávez L. Response to the treatment of brucellosis among children. Evaluation with Huddleson reaction and PCR. Rev Med Inst Mex SeguroSoc. 2007;45(6):615–22.
80. Baddour MM, Alkhalifa DH. Evaluation of three polymerase chain reaction techniques for detection of Brucella DNA in peripheral human blood. Can J Microbiol. 2008;54(5):352–7.
81. Navarro E, Escribano J, Fernández J, Solera J. Comparison of three different PCR methods for detection of Brucella spp. in human blood samples. FEMS Immunol Med Microbiol. 2002;34(2):147–51.
82. Morata P, Queipo-Ortuño MI, de Dios CJ. Strategy for optimizing DNA amplification in a peripheral blood PCR assay used for diagnosis of human brucellosis. J ClinMicrobiol. 1998;36(9):2443–6.
83. Ginzinger DG. Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. ExpHematol. 2002;30(6):503–12.
84. Queipo-Ortuño MI, Colmenero JD, Reguera JM, García-Ordoñez MA, Pachón ME, Gonzalez M, Morata P. Rapid diagnosis of human brucellosis by SYBR Green I-based real-time PCR assay and melting curve analysis in serum samples. ClinMicrobiol Infect. 2005;11(9):713–8.
85. Surucuoglu S, El S, Ural S, Gazi H, Kurutepe S, Taskiran P, Yurtsever SG. Evaluation of real-time PCR method for rapid diagnosis of brucellosis with different clinical manifestations. Pol J Microbiol. 2009;58(1):15–9.
86. Alsayed Y, Monen F. Brucellosis laboratory tests in Syria: what are their diagnostic efficacies in different clinical manifestations? J Infect Dev Ctries. 2012;6(6):495–500.

87. Huber B, Scholz HC, Lucero N, Busse HJ. Development of a PCR assay for typing and subtyping of Brucella species. Int J Med Microbiol. 2009;299(8):563–73.
88. Queipo-Ortuño MI, Colmenero JD, Bermudez P, Bravo MJ, Morata P. Rapid differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis using a multiplex real-time PCR assay. PLoS One. 2009;4(2):e4526.
89. Colmenero JD, Morata P, Ruiz Mesa JD, Bautista D, Bermúdez P, Bravo MJ, Queipo-Ortuño MI. Multiplex real-time polymerase chain reaction: a practical approach for rapid diagnosis of tuberculous and brucellar vertebral osteomyelitis. Spine (Phila Pa 1976). 2010;35(24):E1392–6.
90. Sanjuan Jimenez R, Colmenero JD, Bermúdez P, Alonso A, Morata P. Amplicon DNA melting analysis for the simultaneous detection of Brucella spp. and Mycobacterium tuberculosis complex. Potential use in rapid differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis. PLoS One. 2013;8(3):e58353.
91. Yagupsky P. Detection of Brucellae in blood cultures. J Clin Microbiol. 1999;37(11):3437–42.
92. Araj GF, Lulu AR, Mustafa MY, Khateeb MI. Evaluation of ELISA in the diagnosis of acute and chronic brucellosis in human beings. J Hyg (Lond). 1986;97(3):457–69.
93. Corbel MJ. Brucellosis: an overview. Emerg Infect Dis. 1997;3(2):213–21.
94. Mantur BG, Mangalgi SS. Evaluation of conventional castaneda and lysis centrifugation blood culture techniques for diagnosis of human brucellosis. J Clin Microbiol. 2004;42(9):4327–8.
95. Cetin ES, Kaya S, Demirci M, Aridogan BC. Comparison of the BACTEC blood culture system versus conventional methods for culture of normally sterile body fluids. AdvTher. 2007;24(6):1271–7.
96. Gaviria-Ruiz MM, Cardona-Castro NM. Evaluation and comparison of different blood culture techniques for bacteriological isolation of Salmonella typhi and Brucella abortus. J Clin Microbiol. 1995;33(4):868–71.
97. Gotuzzo E, Carrillo C, Guerra J, et al. Evaluation of diagnostic methods for brucellosis. Value of bone marrow culture. J Infect Dis. 1986;153:122.
98. Diaz R, Moriyon I. Laboratory techniques in the diagnosis of human brucellosis. In: Young EJ, Corbel MK, editors. Brucellosis: clinical and laboratory aspects. Boca Raton: CRC Press; 1989. p. 73.
99. Ozkurt Z, Erol S, Tasyaran MA, Kaya A. Detection of Brucella melitensis by the BacT/Alert automated system and Brucella broth culture. Clin Microbiol Infect. 2002;8(11):749–52.
100. Apa H, Devrim I, Memur S, Gunay I, Gulfidan G, Celegen M, et al. Factors affecting Brucella spp. blood cultures positivity in children. Vector Borne Zoonotic Dis. 2013;13(3):176–80.
101. Deepak S, Bronson SG, Joseph W, Thomas M, Sibi. Brucella isolated from bone marrow. J Assoc Physicians India. 2003;51:717–8.
102. Assadi M, et al. Brucellosis in Iran: a literature review. J Am Sci. 2013;9(3):203–8.
103. Yang B, Hu H, Chen J, He X, Li H. The evaluation of the clinical, laboratory, and radiological findings of 16 cases of Brucellar Spondylitis. Biomed Res Int. 2016;2016:8903635.
104. Farid Z, Trabolsi B, Yassin W, et al. Acute brucellosis presenting as fever of unknown origin (FUO). Trans R SocTropMedHyg. 1980;74:402.
105. Dean AS, Crump L, Greter H, Hattendorf J, Schelling E, Zinsstag J. Clinical manifestations of human brucellosis: a systematic review and meta-analysis. Carabin H, ed. PLoS Negl Trop Dis. 2012;6(12):e1929.
106. Gotuzzo E, Alarcón G, Bocanegra T, et al. Articular involvement in human brucellosis: a retrospective analysis of 304cases. Semin Arthritis Rheum. 1982;12:245–55.
107. Madkour MM. Madkour's brucellosis. Berlin: Springer; 2001.
108. Kennedy JC. Notes on a case of chronic synovitis on bursitis due to tge organism of Mediterranean fever. J Roy Army Med Corps. 1904;2:178–80.
109. Bocanegra T, Gotuzzo E, Alarcón G, et al. Circulating immune complexes in acute fever and brucellosis. Clin Res. 1981;29:381.
110. Gottesman G, Vanunu D, Maayan MC, et al. Childhood brucellosis in Israel. Pediatr Infect Dis J. 1996;15:610.
111. Alarcón GS, Bocanegra T, Gotuzzo E, et al. Reactive arthritis associated with brucellosis: HLA studies. J Rheumatol. 1981;8:621.
112. Bocanegra T, Gotuzzo E, Castañeda O, et al. Rheumatic manifestations of brucellosis. Ann RheumDis. 1986;45:526.
113. Brito M, Gonzales-Dias J, Marques J, et al. Brucellosis osteoarticular. RevEspReumatolEnfermOsteoarthritis (Barcelona). 1972;15:219.
114. Sany P. Una polyarthrite pseudo-rhumatoide. Rhumatologia. 1975;27:411.
115. Hizel K, Guzel O, Dizbay M, et al. Age and duration of disease as factors affectingclinical findings and sacroiliitis in brucellosis. Infection. 2007;35:434–7.
116. Gheita TA, Sayed S, Azkalany GS, et al. Subclinical sacroiliitis in brucellosis. Clinical presentation and MRI findings. Z Rheumatol. 2015;74(3):240–5.
117. Gotuzzo E, Carrillo C. In: Espinoza L, Goldberg D, Arnett F, editors. Infections in the rheumatic disease. Orlando: Grune& Stratton; 1988. p. 31.
118. Norton WL. Brucellosis and rheumatic syndromes in Saudi Arabia. J Rheumatol. 1984;43:810–5.
119. Rotes-Querol J. Manifestaciones osteoarticulares de la Brucelosis. Barcelona, Spain: Barcelona JIMS Editorial; 1959.
120. Feldman JL, Menkes CJ, Weil B, et al. Les sacro-ileitesinfecticuses. Etude multicentriqe sue 214 observations. Rev Rhum Med Osteartic. 1981;48:83–91.
121. Momjian R, George M. Atypical imaging features of tuberculous spondylitis: case report with literature review. JRadiolCaseRep. 2014;8(11):1–14.
122. Ariza J, Gudiol F, Valverde J, et al. Brucellar spondylitis: a detailed analysis based on current findings. Rev Infect Dis. 1985;7:656.
123. Spink WW. The nature of brucellosis. Minneapolis: University of Minnesota Press; 1956.
124. Munoz E. Brucellosis in Kumate J and Gutierrez G: manual de Infectología- Mexico. Edic del Hospital Infantil de Mexico. 1977:32–8.
125. Young EJ. Human brucellosis. Rev Infect Dis. 1983;5:821–42.
126. Ibero I, Vela P, Pascual E. Arthritis of shoulder and spinal cord compression due to. Br J Rheumatol. 1997;36:377.
127. Pons P. Spondylitis melitoccocita. Ann Med. 1929;5:227.
128. Roux H, Peloux Y, Gaborit P, et al. Les manifestations ostéoarticulaires de la brucellose. EncMedChir Paris. 1983;14:1.
129. Turgut M, Turgut A, Koşar U. Spinal brucellosis: Turkish experience based on 452 cases published during the last century. Actaneurochirurgica. 2006;148(10):1033–44.
130. Bozgeyik Z, Ozdemir H, Demirdag K, Ozden M, Sonmezgoz F, Ozgocmen S. Clinical and MRI findings of brucellar spondylodiscitis. Eur J Radiol. 2008;67(1):153–8.
131. Tekkok IH, Berker M, Ozcan OE, et al. Brucellosis of the spine. Neurosurgery. 1993;33:838–44.
132. Iqbal QM, Khan O. Brucellosis of the spine. J R CollSurgEdinb. 1990;35:395–7.
133. Tu L, Liu X, Gu W, et al. Imaging-assisted diagnosis and characteristics of suspected spinal brucellosis: aretrospective study of 72 cases. Med Sci Monit. 2018;24:2647–54.
134. Arslan F, Karagoz E, Arslan BY, et al. Spinal brucellosis diagnosed with positron emission tomography combined with computed tomography (PET/CT). Spine J. 2016;16:e381–2.

135. Lim K-B, Kwak Y-G, Kim D-Y, Kim Y-S, Kim J-A. Back pain secondary to Brucella spondylitis in the lumbar region. Ann Rehabil Med. 2012;36(2):282–6.
136. Cobbaert K, Pieters A, Devinck A, Devos M, Goethals I, Mielants H. Brucellarspondylodiscitis: case report. Acta Clin Belg. 2007;62(5):304–7.
137. Lynch EC, McKechnie JC, Alfrey CP. Brucellosis with pancytopenia. Ann Intern Med. 1968;69:319.
138. Uluğ M, Yaman Y, Yapici F, et al. Clinical and laboratory features complications and treatment outcome of brucellosis in childhood and review of the literature. Turk J Pediatr. 2011;53(4):413–24.
139. Eser B, Altuntas F, Soyuer I, et al. Acute lymphoblastic leukemia associated with brucellosis in two patients with fever and pancytopenia. YonseiMed J. 2006;47(5):741–4.
140. Navarro-Martínez A, Solera J, Corredoira J, et al. Epididymoorchitisdueto. ClinInfectDis. 2001;33:2017.
141. Al Deeb SM, Yaqub BS, Sharif HS, et al. Neurobrucellosis: clinical characteristics, diagnosis and outcome. Neurology. 1989;39:498.
142. Bashir R, Al-Kawl Z, Harder EJ, et al. Nervous system brucellosis: diagnosis and treatment. Neurology. 1985;35:1576.
143. Bouza E, de la Torre MG, Parras F, et al. Brucellar meningitis. RevInfectDis. 1987;9:810.
144. Karaoglan I, Namiduru M, Akcali A, Cansel N. Different manifestations of nervous system involvement by neurobrucellosis. Neurosciences. 2008;13:283–7.
145. Elberg SS. A guide to the diagnosis, treatment and prevention of brucellosis. Bull WHO. 1981;81:31.
146. Bruguera M, Cervantes F. Hepatic granulomas in brucellosis. Ann Intern Med. 1980;92:571.
147. Spink WW, Hoffbauer FW, Walker WW, et al. Histopathology of the liver in human brucellosis. J Lab Clin Med. 1949;34:40.
148. Arias Stella J. Brucellosis. Contribución al conocimiento patológico. Ann FacMed (Peru). 1951;34:429.
149. El-Koumi MA, Afify M, Al-Zahrani SH. A prospective study of brucellosis in children: relative frequency of pancytopenia. Iran J Pediatr. 2014;24(2):155–60.
150. Al-Anazi KA, Al-Jasser AM. Brucella bacteremia in patients with acute leukemia: a case series. J Med Case Rep. 2007;1:144.
151. Mantur BG, Amarnath SK, Shinde RS, et al. Review of clinical and laboratory features of human brucellosis. Indian J MedMicrobiol. 2007;25(3):188–202.
152. Al Hashan GM, el-Fetoh NMA, Nasser IA, et al. Pattern of childhood brucellosis in Najran, south Saudi Arabia in 2013–2017. Electron Physician. 2017;9(12):5902–7. https://doi.org/10.19082/5902.
153. Sari I, Altuntas F, Hacioglu S, et al. A multicenter retrospective study defining the clinical and hematological manifestations of brucellosis and pancytopenia in a large series: hematological malignancies, the unusual cause of pancytopenia in patients with brucellosis. Am J Hematol. 2008;83(4):334–9.
154. Serpa JA, Knights S, Farmakiotis D, Campbell J. Brucellosis in adults and children: a 10-year case series at two large Academic Hospitals in Houston, Texas. SouthMed J. 2018;111(6):324–7.
155. Solera J, Martinez-Alfaro E, Espinosa A. Recognition and optimum treatment of brucellosis. Drugs. 1997;53:245–56.
156. Salvana EMT, Salata RA. Brucellosis. In: Goldman L, Schafer AI, editors. Goldman-Cecil medicine. Philadelphia: Elsevier-Saunders; 2016.
157. Spink WW, Braude AI, Castaneda MR, et al. Aureomycin therapy in human brucellosis due to Brucella melitensis. JAMA. 1948;138:1145–8.
158. Baykam N, Esener H, Egonul O, Eren S, Celikbas AK, Dokuzoguz B. In vitro antimicrobial susceptibility of Brucella species. Int J Antimicrob Agents. 2004;23:405–7.
159. Dimitrov TS, Panigrahi D, Emara M, Awni F, Passadilla R. Seropidemiological and microbiological study of brucellosis in Kuwait. Med PrincPract. 2004;13:215–9.
160. Ariza J, Gudiol F, Pallares R, et al. Comparative trial of rifampicin/doxycycline versus tetracycline/streptomycin in the therapy of human brucellosis. Antimicrob Agents Chemother. 1985;28:548–51.
161. Llorens-Terol J, Busquets RM. Brucellosis treated with rifampin. Arch Dis Child. 1980;55:486–8.
162. Acocella G, Bertrand A, Beytout J, et al. Comparison of the three different regimens in the treatment of acute brucellosis: a multicenter study. J AntimicrobChemother. 1989;23:433–9.
163. Waitz JA, Weinstein MJ. Recent antimicrobiological studies with gentamicin. J InfectDis. 1969;119:355–60.
164. Joint FAO/WHO expert committee on brucellosis. World Health Organ Tech Rep Ser. 1986;740:1–132.
165. Ariza J, Bosilwowski M, Cascio A, Colmenero JD, Corbel MJ, Falagas ME, et al. Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PLoSMed. 2007;4:1872–8.
166. Solera J, Martinez-Alfaro E, Saez L. Meta-analysis of the efficacy of the combination of rifampicin and doxycycline in the treatment of human brucellosis. Med Clin (Barc). 1994;102:731–8.
167. Colmenero Castillo JD, HernandezMarquez S, Reguera Iglesias JM, Cabrera Franquelo F, RiusDiaz F, et al. Comparative trial of doxycycline plus streptomycin versus doxycycline plus rifampin for the therapy of human brucellosis. Chemotherapy. 1989;35:146–52.
168. Cisneros JM, Viciana P, Colmenero J, Pachon J, Martinez C, et al. Multicenter prospective study of treatment of Brucella melitensis brucellosis with doxycycline for 6 weeks plus streptomycin for 2 weeks. Antimicrob Agents Chemother. 1990;34:881–3.
169. Ariza J, Gudiol F, Pallares R, Viladrich PF, Rufi G, et al. Treatment of human brucellosis with doxycycline plus rifampin or doxycycline plus streptomycin. A randomized, double-blind study. Ann Intern Med. 1992;117:25–30.
170. Solera J, Rodríguez-Zapata M, Geijo P, et al. Doxycycline-rifampin versus doxycycline-streptomycin in treatment of human brucellosis. AntimicrobAgentsChemother. 1995;39:2061–7.
171. Colmenero JD, Fernandez-Gallardo LC, Agundez JA, Sedeno J, Benitez J, et al. Possible implications of doxycycline-rifampin interaction for treatment of brucellosis. Antimicrob AgentsChemother. 1994;38:2798–802.
172. Garraffo R, Dellamonica P, Fournier JP, Lapalus P, Bernard E, et al. Effects of rifampicin on the pharmacodynamics of doxycycline. PatholBiol (Paris). 1987;35:746–9.
173. Marianelli C, Ciuchini F, Tarantino M, Pasquali P, Adone R. Genetic bases of the rifampin resistance phenotype in Brucella spp. J ClinMicrobiol. 2004;42:5439–43.
174. Pappas G, Siozopoulou V, Akritidis N, Falagas ME. Doxycycline-rifampicin: Physicians' inferior choice in brucellosis or how convenience reigns over science. J Infect. 2007;54:459–46.
175. Pappas G, Siozopoulou V, Saplaoura K, Vasiliou A, Christou L, et al. Health literacy in the field of infectious diseases: the paradigm of brucellosis. J Infect. 2007;54:40–5.
176. World Health Organization. Brucellosis in humans and animals. Geneva: WHO; 2006. WHO/CDS/EPR/2006.7.
177. Akova M, Uzun O, Akalin HE, Hayran M, Unal S, Gur D. Quinolones in treatment of human brucellosis: comparative trial of ofloxacin- rifampin versus doxycycline-rifampin. Antimicrob Agents Chemother. 1993;37:1831–4.
178. Karabay O, Sencan I, Kayas D, Sahin I. Ofloxacin plus rifampicin versus doxycycline plus rifampicin in the treatment of brucellosis: a randomized clinical trial. BMC Infect Dis. 2004;4:18.
179. Saltoglu N, Tasova Y, Inal AS, Seki T, Aksu HS. Efficacy of rifampicin plus doxycycline versus rifampicin plus quinolone in the treatment of brucellosis. Saudi Med J. 2002;23:921–4.
180. Kalo T, Novi S, Nushi A, Dedja S. Ciprofloxacin plus doxycycline versus rifampicin plus doxycycline in the treatment of acute brucellosis. Med Mal Infect. 1996;26:587–9.

181. Agalar C, Usubutun S, Turkyilmaz R. Ciprofloxacin and rifampicin versus doxycycline and rifampicin in the treatment of brucellosis. Eur J ClinMicrobiol Infect Dis. 1999;18:535–8.
182. Skalsky K, Yahav D, Bishara J, Pitlik S, Leibovici L, Paul M. Treatment of human brucellosis: systematic review and meta-analysis of randomized controlled trials. BMJ. 2008;336:701–4.
183. Hashemi SH, Gachkar L, Keramat F, Mamani M, et al. Comparison of doxycycline- streptomycin, doxycycline-rifampin, and ofloxacin-rifampin in the treatment of brucellosis: a randomized clinical trial. Int J Infect Dis. 2012;16:e247–51.
184. Pappas G, Christou I, Akritidis N, Tsianos EV. Quinolones for brucellosis: treating old diseases with new drugs. ClinMicrobiol Infect. 2006;12:823–5.
185. Hasanain A, Mahdy R, Mohamed A, Mostafa A. A randomized, comparative study of dual therapy (doxycycline- rifampin) versus triple therapy (doxycycline- rifampin- levofloxacin) for treating acute/subacute brucellosis. Braz J Infect Dis. 2016;20(3):250–4.
186. Solera J, Martinez-Alfaro E, Espinoza A, Castillejos MI, Geijo P, Rodríguez-Zapata M. Multivariate model for predicting relapses in human brucellosis. J Infect. 1998;36:85–92.
187. Raoult D, Houpikian P, TissotDupont H, Riss JM, Arditi-Djiane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Intern Med. 1999;159:167–73.
188. Majzoobi MM, Hashemi SH, Mamani M, Keramat F, Poorolajal J, Basir HRG. Effect of hydroxychloroquine on treatment and recurrence of acute brucellosis: a single-blind randomized clinical trial. Int J Antimicrob Agents. 2018;51(3):365–9.