Neurobrucellosis

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Introduction

Brucellosis, also known as "undulant fever," "Mediterranean fever," or "Malta fever," is an important human zoonosis and a major public health issue in many parts of the world especially in the Mediterranean countries of Europe, North and East Africa, the Middle East, South and Central Asia, and Central and South America [1].

Among Mediterranean countries, it has been reported that Syria has 16,034 cases followed by Iraq (2784), Turkey (2622), and the Kingdom of Saudi Arabia (KSA) (2144) per million population [2]. *Brucella* are aerobic gram-negative intracellular coccobacilli, four species of which are known to cause disease in humans, namely, *B. melitensis*, *B. suis*, *B. canis*, and *B. abortus*. More recently, marine mammals have been recognized as additional animal reservoirs for *Brucella* species with zoonotic potential. *B. ceti* and *B. pinnipedialis* are the newly proposed species names.

The most severe form is caused by *B. melitensis* that is predominant in KSA and the Middle East. It is transmitted from animals indirectly via consumption of raw milk and milk products, butchering of raw meat or directly by contact with livestock (sheep, goat, camels), milking, and handling parturient of animals such as contact with placenta membrane. In Al Medina region alone, the prevalence of brucellosis was 2.6% and was shown to increase with age in rural communities and low socioeconomic status. The overall prevalence of brucellosis among livestock as assessed by examining blood from a random sample of animals was estimated at 17.4% [3]. A recently published study from KSA reported a significant reduction of incidence rate from 22.9 in 2004 to 12.5 in 2012 per 100,000 persons for the total population [4].

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Clinical Manifestation

Brucellosis can involve any system of the body including the central nervous system.

Most of the studies report that an element of CNS is involved in 4–13% of brucellosis patients [5].

Neurobrucellosis (NB) is defined as isolation of *Brucella* species from CSF of patients with suspected findings for brucellosis, or isolation of *Brucella* species from bone marrow or blood cultures of patients with abnormal CSF findings, with or without standard tube agglutination (STA) positivity of any titer in CSF with abnormal findings [6].

In another series of 128 patients with laboratory-confirmed brucellosis and neurological signs and symptoms, 48 (37.5%) were diagnosed with NB according to any one of the following diagnostic criteria: (1) symptoms and signs suspecting NB; (2) isolation of *Brucella* species from cerebrospinal fluid (CSF) and/or presence of anti-*Brucella* antibodies in CSF; (3) the presence of lymphocytosis, increased protein, and decreased glucose levels in the CSF; or (4) findings in cranial magnetic resonance imaging (MRI) or computed tomography (CT) [7].

Neurobrucellosis is a rare but severe complication occurring in about 5% of systemic brucellosis. It poses a diagnostic challenge, often resembling a variety of other neurologic disorders.

It can manifest in various forms, the most common being meningitis and/or meningoencephalitis-meningomyelitis (acute, subacute, or chronic) and polyradiculoneuropathy with or without cranial nerve involvement (most often the eighth nerve) which is subacute or chronic. Cerebrovascular accidents may present in the following ways: transient ischemic attacks; occlusive episodes; venous thrombosis, either sudden or progressive; thrombophlebitis of the brain and the eye; or subarachnoid and intracerebral hemorrhage due to rupture of mycotic aneurysms.

The clinical picture may be much more subtle and often deceptive, resembling demyelinating, multisystem degenerative, and other localized or diffuse central and/or peripheral nervous system disorders. A patient with recurrent episodes of diplopia and pyramidal symptoms would most likely direct diagnostic probabilities toward multiple sclerosis, which is rampant in Saudi Arabia. Similarly, a young patient with slowly progressive ataxia, polyradiculoneuropathy, and deafness is most likely to suffer from a degenerative, probably inherited disease or may present with Guillain-Barre syndrome. An obese young lady with papilledema and sixth nerve palsy may suffer from benign intracranial hypertension, but CSF shows findings of chronic meningitis, without overt clinical evidence.

In another young patient, with transient and recurrent alternating hemiplegia, NB would be the most implausible choice in the diagnostic list of the experienced neurologist. In addition, a patient with NB may appear with acute confusional episodes or a motor neuron disease-like syndrome or a unilateral brachial neuropathy reminiscent of neuralgic amyotrophy. Other more common presentations may be due to spinal nerve root and/or cord involvement secondary to spinal disc and bone infection (spodylodiscitis) [8].

Studies from Saudi Arabia show that approximately half of clinically diagnosed brucellosis patients have osteoarticular involvement with sacroiliitis, peripheral arthritis, and destructive spondylitis as common presentations [9]. Clinical manifestations and CSF abnormality are similar to tuberculosis, and NB must be kept in mind when approaching patients with acute or chronic lymphocytic meningitis with increased protein and low glucose level in CSF and risk factors of brucellosis [10].

Thwaites and Lancet scoring systems are widely used to aid clinicians practicing in resource-poor countries to predict TB meningitis. Since *Brucella* meningoencephalitis is clinically and biochemically indistinguishable from TB meningitis, the validity of Thwaites and Lancet prediction scoring systems was assessed in a large retrospective Turkish cohort where 294 confirmed *Brucella* meningoencephalitis patients were compared to 190 cases of confirmed TB meningitis selected from Hydarpasa studies database. Interestingly those scoring systems have falsely identified *Brucella* meningoencephalitis patients as TB meningitis; therefore, the authors concluded that *Brucella* meningoencephalitis should be excluded by every diagnostic microbiologic modality when such prediction systems suggest TB meningitis [11].

Further, it is important to think about the diagnosis of NB in patients with subacute-chronic and obscure neurologic involvement, especially living in endemic regions, because NB may potentially cause irreversible neurologic disability.

Diagnosis

The diagnosis of brucellosis is based on serological and microbiological laboratory tests. Full blood count would reveal normal to low leukocyte counts. Minor changes in liver enzymes are noticeable [12]. CSF shows pleocytosis with predominant mononuclear cells. Elevated CSF adenosine deaminase (ADA) is suggestive of *Brucella* meningitis but may also indicate TB meningitis. In a study by Karsen et al., the mean ADA values in CSF of TB meningitis cases were 28.34 compared to 8.71 IU/L in *Brucella* meningoencephalitis. A cutoff value of 12.5 IU/L for the differential diagnosis of TB versus *Brucella* meningoencephalitis has a sensitivity of 92% and a specificity of 88% [13].

In *Brucella* arthritis, leukocytosis with lymphocytic predominance is dominant [12].

Microbial culture is the ideal method in making a diagnosis of brucellosis by culturing the organism from blood, bone marrow, liver biopsy specimen, and/or other body fluids or tissues [14, 15].

Serological tests detect antibodies to the antigens of *Brucella* species in blood. The antigens include smooth lipopolysaccharide (S-LPS) and cytosolic protein. The serological tests such as *serum agglutination testing (SAT)* and enzyme-linked immunosorbent assay (ELISA) detect antibodies against the S-LPS antigen [16].

The Rose Bengal test (RBT) is a rapid, slide-type agglutination assay performed with a stained *Brucella abortus* suspension at a low pH (3.6–3.7) and plain serum. It is a simple and ideal screening test for small laboratories with limited resources that is based on reactivity of antibodies against smooth lipopolysaccharide (LPS)

[17]. *The microagglutination test (MAT)* is a variant of the SAT or ELISA recommended for the serodiagnosis of brucellosis that is rapid and requires less volumes of serum and reagents (antigen and serum) than SAT and can test multiple samples at the same time but has high false-negative rates in complicated and chronic cases.

Coombs test is good for complicated and chronic cases but misses about 7% of cases compared with ELISA [18].

Dipstick assay is a good test to detect IgM antibodies to S-LPS in brucellosis of less than 3 months duration. IgM dipstick assay offers higher sensitivity and easier manipulation than IgM ELISA to detect IgM antibodies to *Brucella* species and improves the interpretation of results, thus establishing cutoff points. It could be used as a rapid and simple alternative to the ELISA IgM for the serodiagnosis of patients with acute brucellosis. The combined results of SAT and IgM dipstick assays can provide an indication of the stage of disease for those patients, in whom the onset of clinical manifestations is unknown [19].

The rapid slide agglutination test (RSAT) could be a suitable screening test for the diagnosis of *B. canis* human brucellosis, and a supplementary technique, such as ELISA, performed on all positive RSAT samples that were negative by *B. abortus* antigen could ensure diagnostic specificity and confirm the diagnosis [20]. Immunochromatographic *Brucella* IgM/IgG lateral flow assay (LFA), a simplified version of ELISA, has a great potential as a rapid point-of-care assay. It has high sensitivity and specificity for *Brucella* IgM and IgG. It is a rapid and simple diagnostic test for confirmation of brucellosis in an endemic area [21].

New Brucella markers can be detected by flow cytometry on $CD4^+$ and $CD8^+$ cells in seronegative patients with brucellosis that can be utilized as a novel diagnostic test for the detection of brucellosis in seronegative individuals [22].

Brucella immunocapture-agglutination test (Brucellacapt), which is based on sandwich ELISA system, is performed with Coombs antiserum and determines the three antibodies that form against *Brucella* (IgM, IgA, IgG). The advantage of this test is that it shows existence of blocking antibodies that is a reason for a false negative test by SAT and RBT. At a cutoff value of 1/160 and 1/320, Brucellacapt sensitivity is 95–100% and has a specificity of 55–59%. It is useful to diagnose disease in patients with long-standing evolution of brucellosis and in the follow-up of treatment; therefore it is considered as a second-level serological test [23].

Molecular Diagnosis of Brucellosis

Standard PCR has excellent sensitivity for the diagnosis of acute and relapsed cases of brucellosis where serology is often negative [19, 24]. It can be applied on blood, serum, or synovial fluid. The standard PCR assays include one pair of primers which is used to amplify the target genomic sequence of *Brucella* spp. Pairs used include the primers for sequences encoding 16S rRNA, outer membrane protein (omp2a, omp2b, and omp31), 31 kDa immunogenic *Brucella abortus* protein (BCSP 31 B4/B5), 16S–23S ribosomal DNA interspace region (ITS66/ITS279), and insertion sequence (IS711).

Real-time PCR seems to be highly reproducible, rapid (final result in 30 min), sensitive, and specific. Additionally, the risk of infection in laboratory workers is minimal. Samples that have been tested by real-time PCR include cultured *Brucella* cells, serum, blood, and paraffin-embedded tissues. The IS711-based assay was the most sensitive, specific, efficient, and reproducible method to detect *Brucella* spp.

Several multiplex PCRs have been reported which identify the genus *Brucella* at the species and partly at the biovar level using different primer combinations [21, 24, 25].

It has a great utility in chronic and atypical cases. The most interesting use of multiplex PCR is that it simultaneously detects *Brucella* spp. and *Mycobacterium tuberculosis* complex in countries where both diseases are endemic. The procedure targeted the IS711, bcsp31, and omp2a genes for *Brucella* spp. and the IS6110, senX3-regX3, and cfp31 genes for *M. tuberculosis* complex.

Angiography is used for detection of vascular changes. *Neurophysiologic electromyographic* and *nerve conduction* studies are reserved for cases with peripheral and cranial nerve involvement [26].

Neuroimaging

A recent multicenter study has evaluated 263 adults with NB and reviewed their CT and MRI images. They categorized the finding into five groups. Group 1 had normal CT and MRI (143 patients, 54.3%), and group 2 had inflammatory changes (72 patients, 27.4%), diffuse inflammation (59 patients) including leptomeningeal involvement (44 patients), basal meningeal enhancement (30 patients), and localized inflammation (24 patients), in the form of cranial nerve involvement (14 patients), spinal nerve root enhancement (8 patients), brain abscess (7 patients), granuloma (6 patients), and arachnoiditis (4 patients); 11 patients had co-existent diffuse inflammation. Group 3 had white matter abnormalities (32 patients, 12.2%) and demyelinating lesions (7patients), while group 4 had vascular insults (42 patients, 16%), of which 37 patients had chronic cerebral ischemic changes, two patients had acute cerebral ischemia, two had subdural hematomas, and one patient had a subarachnoid hemorrhage. Group 5 had cerebral edema/hydrocephalus (48 patients, 18.2%), and 20 patients (7.6%) had hydrocephalus; cerebral edema was seen in 40 out of 263 patients (15%), while coexistent cerebral edema and hydrocephalus were seen in 12 patients. The authors concluded that diffuse inflammation is the primary neuroimaging abnormality which is most commonly seen with longer duration of symptoms, higher CSF protein, lower CSF/serum glucose ratio, and with the presence of polyneuropathy or radiculopathy on clinical examination [27].

Focal cord expansion and poorly delineated increased signal in spinal cord on T2 W images may be seen in case of myelopathy due to involvement of spinal cord. In *Brucella* spondylitis, the lumbar spine is the most commonly involved site, particularly the L4–L5 and L5–S1 junctions. In the majority of patients (98%), a solitary lesion was identified. However, the incidence of multiple site involvement has been reported as high as 9–30% in some studies. Abscess formation has become a

common finding (21–42%) following the development of highly sensitive diagnostic techniques such as CT and MRI [26].

Among 20 patients with spondylodiscitis, it was complicated with paravertebral or epidural abscess in seven, radiculitis in six, and psoas abscess in five of cases [28, 29]. The demonstration of IgG/oligoclonal bands in CSF and serum is a rapid test which can be used as an important index in the diagnosis of NB at the time of presentation, as it may be confused with CNS infections with mycobacteria, treponema, or fungi [30].

Treatment and Prevention Challenges

The optimal drug treatment and duration are both controversial. The treatment of central nervous system complications of brucellosis poses a special problem because of the need to achieve high concentrations of drugs in the CSF. Although doxycycline is the best among tetracyclines in penetrating the blood-brain barrier, it is recommended to add other drugs which achieve this, such as rifampicin or cotrimoxazole in the treatment regimen of patients with NB [1].

Some studies showed the benefit of adding third-generation cephalosporin such as ceftriaxone in NB as it achieves concentrations in CSF higher than the MIC against *Brucella* species. In the Istanbul study, adult patients treated for NB were retrospectively reviewed in 28 healthcare institutions from four different countries. It was found that ceftriaxone-based regimens are more successful in terms of less clinical failure and relapse, and they require shorter therapy than the oral treatment protocol alone [31].

The usual span of treatment is as short as 6–8 weeks up to 18 months if patients have residual disease [32].

Although adding steroids in NB has not been proved to be consistently beneficial, adjunctive corticosteroid therapy has been used for concurrent vasculitis or demyelinating disease [33].

In a series of patients with spondylitis, antibiotic regimens included two or three antibiotics with combination of doxycycline, rifampin, and streptomycin. The mean duration of antimicrobial therapy was 18 weeks (range 12–56 weeks). Prolonged duration of treatment is important especially in complicated cases in order to avoid possible sequelae [28, 30].

Doxycycline, 100 mg twice daily, for at least 12 weeks combined with streptomycin, 1 g daily, for the first 2 or 3 weeks remains the first choice of antibiotic therapy in *Brucella* spondylitis [29, 30]. The use of streptomycin in CNS brucellosis is discouraged owing to its questionable ability to penetrate into the cerebrospinal fluid and its potential neurotoxicity that may perplex the clinical presentation [32, 33].

The Saudi Pediatric Infectious Diseases Society recommends treating NB in children above 8 years with doxycycline and trimethoprim-sulfamethoxazole (TMP-SMX), while for those younger than 8 years, rifampicin, TMP-SMX, and ciprofloxacin for 3–6 months up to 1 year in complicated cases. Gentamicin is added in the initial 14 days with the option of adding ceftriaxone in the initial 2–4 weeks [34].

There are no randomized trials for brucellosis in pregnancy. The most extended series support the use of TMP-SMX alone or in combination with rifampicin [35].

Surgical intervention should be carried out in NB if indicated as in other CNS infections. The challenge lies in establishing guidelines for diagnosis and treatment as each case is unique and the clinical manifestations vary from individual to individual. Not all forms of NB are the same nor they carry a similar prognosis. Relapses are also not unusual. Further adverse effects due to drug therapy or due to the complications of the disease itself needs careful monitoring over a period of time. Early clinical and laboratory diagnosis followed by ideal and prompt treatment for adequate period of time is indispensable to prevent lifelong residual deficits.

Establishment of National Brucellosis control program is recommended not only for KSA but also for all endemic regions. Animal husbandry should be properly practiced.

Detailed information on frequency and distribution of infection is required to estimate cost effective options for control.

Consumption of raw milk should be avoided in all age groups until regular screening services can be provided.

Strategic vaccination of ruminants combined with public health education programs may help in controlling the disease.

Through national and international collaboration well-designed epidemiological studies should be conducted to bridge the gap in the management of brucellosis [36].

Importation of *Brucella* spp. especially into non-endemic areas, or areas which have achieved recent control of both animal and human brucellosis, may have public health repercussions, and timely recognition is essential [37]. In pediatric brucellosis cases, family history has been reported in 33% of cases in Turkey. So screening of family members when a patient with brucellosis is diagnosed is very important [38]. Effective vaccines are currently available and it is important to find means and resources for their effective use in resource-poor countries in conjunction with sustained control efforts that incorporate local farming practices, dietary habits, and traditional beliefs [39].

Mixing different herds of animals together should be avoided as this practice facilitates the transmission of disease among animals. The government should stress the screening of animals, the vaccination of seronegative animals, and slaughtering diseased ones. A collaborative team to implement a brucellosis control program should be arranged and maintained among the concerned government sectors including the Ministry of Health, the Ministry of Agriculture, the Custom Department, and the Municipal Department [34].

Indeed, with such extraordinary advancement in healthcare system and general awareness, brucellosis should be eradicated from this region.

Conclusion

Zoonotic brucellosis remains widespread and neglected in many areas despite notable advances in science, technology, and management in the nineteenth and twentieth centuries [40].

Neurobrucellosis is not readily identified because of its variable picture and must be prioritized in the list of differential diagnosis of any neurological disorder in patients living in or returning from endemic area.

Diagnosis depends on keen awareness of possible infection and a thorough occupational and travel history. A definitive diagnosis requires isolation of the organism by culture of blood, CSF, bone marrow, or other clinical samples. However, a diagnosis of brucellosis is often made serologically, most frequently by standard tube agglutination measuring antibody to *B. abortus* antigen or ELISA, which is more sensitive and specific. The mortality rate of brucellosis is very low (0.1%) and is associated with late diagnosis and late therapy, especially when *Brucella* affects the central nervous system, resulting in meningitis or cerebral abscess. Therapeutic intensity is obviously higher in focal disease, some cases requiring surgery and/or a longer duration of antibiotic therapy. Combination antimicrobial therapy with more than two agents for a prolonged duration that may extend to 6–9 months is necessary to control NB and prevent relapse.

Patients with persistent symptoms following extended antibiotic therapy, for whom focal disease or relapse have been ruled out pose a difficult clinical management problem. This disabling syndrome, sometimes called chronic brucellosis, is similar to chronic fatigue syndrome and must be treated symptomatically [41].Since there is no human vaccine and no significant human-to-human transmission, control of animal brucellosis, milk pasteurization, and other food hygiene measures are the only options to reduce its occurrence in humans. The challenges and opportunities for brucellosis management must be recognized as fundamentally multivariate, multifaceted, and integrative; it is crucial for veterinary, public health, and wildlife/conservation professions to collaboratively develop, adopt and declare brucellosis one health paradigm [40].

References

1. Corbel MJ, et al. Brucellosis in humans and animals. WHO/CDS/EPR/2006.7.
2. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis. 2006;6(2):91–9.
3. Al-Sekait MA. Epidemiology of brucellosis in Al Medina region, Saudi Arabia. J Fam Community Med. 2000;7(1):47–53.
4. Aloufi A, Memish Z, Assiri M, McNabb S. Trends of reported human cases of brucellosis, Kingdom of Saudi Arabia, 2004–2012. J Epidemiol Glob Health. 2016;6:11–8.
5. Alhedaithy A, Aldubayan N, Alqurashi D, Aldubayan S. Clinical-features, serological patterns and long-term complications of 22 patients with neurobrucellosis. Int J Adv Res. 2016;4(10):1682–9.
6. Buzgan T, Karahocagil MK, Irmak H, Baran AI, Evirgen O, Akdeniz H. Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. Int J Infect Dis. 2010;14:e469–78.
7. Guven T, Ugurlu K, Ergonul O, Celikbas AK, Gok SE, Comoglu S, et al. Neurobrucellosis: clinical and diagnostic features. Clin Infect Dis. 2013;56(10):1407–12.
8. Panayiotopoulos CP. Neurobrucellosis: a cause for concern and action. Ann Saudi Med. 1988;8(30):167–8.

9. Paul E, Abdelkareem M, Malik S. Overview of human brucellosis in Aseer region, Saudi Arabia. AMJ. 2017;10(3):202–10.
10. Pourhassan A. Clinical and laboratory findings in neurobrucellosis: a study of 43 cases. Iran J Clin Infect Dis. 2007;2(2):71–6.
11. Erdem H, Senbayrak S, Gencer S, Hasbun R, Karahocagil M, Sengoz G. Tuberculous and brucellosis meningitis differential diagnosis. Travel Med Infect Dis. 2015;13(2):185–91.
12. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. N Engl J Med. 2005;352:2325.
13. Karsen H, Koruk S, Karahocagil M, Calisir C, Baran F. Comparative analysis of cerebrospinal fluid adenosine deaminase activity in meningitis. Swiss Med Wkly. 2011;141:w13214.
14. Young EJ. Brucellosis: current epidemiology, diagnosis, and management. Curr Clin Top Infect Dis. 1995;15:115.
15. Aliskan H. The value of culture and serological methods in the diagnosis of human brucellosis. Mikrobiyol Bul. 2008;42:185–95.
16. Shenoy B, Jaiswal A, Vinod A. Lab diagnosis of brucellosis. Pediatr Infect Dis. 2016;8:40–4.
17. Serra J, Viñas M. Laboratory diagnosis of brucellosis in a rural endemic area in northeastern Spain. Int Microbiol. 2004;7:53.
18. Araj GF. Update on laboratory diagnosis of human brucellosis. Int J Antimicrob Agents. 2010;36:12–7.
19. Geresu MA, Kassa GM. A review on diagnostic methods of brucellosis. J Vet Sci Technol. 2016;7:323.
20. Lucero NE, Escobar GI, Ayala SM, Jacob N. Diagnosis of human brucellosis caused by Brucella canis. J Med Microbiol. 2005;54:457–61.
21. Christopher S, Umapathy BL, Ravikumar KL. Brucellosis: review on the recent trends in pathogenicity and laboratory diagnosis. J Lab Physicians. 2010;2:55–60.
22. Sun Y-H, Rolan HG, den Hartigh AB, Sondervan D, Tsolis RM. Brucella abortus VirB12 is expressed during infection but is not an essential component of the type IV secretion system. Infect Immun. 2005;73:6048–54.
23. Özdemir M, Feyzioglu B, Kurtoglu MG, et al. A comparison of immunocapture agglutination and ELISA methods in serological diagnosis of brucellosis. Int J Med Sci. 2011;8(5):428–32.
24. Andriopoulos P, Tsironi M. Molecular diagnosis of brucellosis: a brief report. Adv Mol Diag. 2016;1:108.
25. Sanjuuan-Jimenez R, Colmenero J, Bermudez 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 complication of brucellosis. PLoS One. 2013;8(3):e58353.
26. Kizilkilic O. Neurobrucellosis. Neuroimaging Clin N Am. 2011;21:927–37.
27. Erdem H, Senbayrak S, Meriç K, Batirel A, Karahocagil MK, Hasbun R, et al. Cranial imaging findings in neurobrucellosis: results of Istanbul-3 study. Infection. 2016;44(5):623–3127.
28. Ulu-Kilic A, Sayar MS, Tutuncu E, Sezen F, Sencan I. Complicated brucellar spondylodiscitis: experience from an endemic area. Rheumatol Int. 2013;33(11):2909–12.
29. Alp E, Doganay M. Current therapeutic strategy in spinal brucellosis. Int J Infect Dis. 2008;12:573–7.
30. Daif AK. The value of oligoclonal bands in neurobrucellosis. Ann Saudi Med. 1991;11(4):411–3.
31. Erdem H, Ulu-Kilic A, Kilic S, et al. Efficacy and tolerability of antibiotic combinations in neurobrucellosis: results of the Istanbul study. Antimicrob Agents Chemother. 2012;56(3):1523–8.
32. Pappas G, Akritidis N, Christou L. Treatment of neurobrucellosis: what is known and what remains to be answered. Expert Rev Anti Infect Ther. 2007;5(6):983–90.
33. Madjdinasab N, Naieni AR. Neurobrucellosis and steroid therapy. Iran J Clin Infect Dis. 2006;1(2):103–7.
34. Alshaalan MA, Alalola SA, Almuneef M, Albanyan EA, Balkhy HH, AlShahrani DA, AlJohani S. Brucellosis in children: prevention, diagnosis and management guidelines for general pediatricians endorsed by the Saudi Pediatric Infectious Diseases Society (SPIDS). Int J Pediatr Adolesc Med. 2014;1:40e46.

35. Ariza J, Bosilkovski M, Cascio A, et al. Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PLoS Med. 2007;4(12):e317.
36. Musallam I, Abo-Shehada M, Hegazy Y, Holt H, Guitian F. Systematic review of brucellosis in the Middle East: disease frequency in ruminants and humans and risk factors for human infection. Epidemiol Infect. 2016;144(4):671–85.
37. Norman FF, Monge-Maillo B, Chamorro-Tojeiro S, Pérez-Molina JA, Lopez-Velez R. Imported brucellosis: a case series and literature review. Travel Med Infect Dis. 2016;14(3):182–99.
38. Yoldas T, Tezer H, Ozkaya-Parlakay A, Sayli TR. Clinical and laboratory findings of 97 pediatric brucellosis patients in Central Turkey. J Microbiol Immunol Infect. 2015;48:446–9.
39. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. Lancet Infect Dis. 2007;7:775–6.
40. Plumb GE, Olsen SC, Buttke D. Brucellosis: 'one health' challenges and opportunities. Rev Sci Tech. 2013;32(1):271–8.
41. Solera J. Update on brucellosis: therapeutic challenges. Int J Antimicrob Agents. 2010;36(Suppl 1):S18–20.