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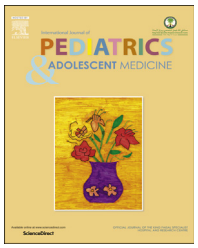

CLINICAL PRACTICE GUIDELINES

Brucellosis in children: Prevention, diagnosis and management guidelines for general pediatricians endorsed by the Saudi Pediatric Infectious Diseases Society (SPIDS)

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Abstract In Saudi Arabia, brucellosis is an endemic zoonotic disease. Although it is believed that children are not commonly involved, a number of reports from endemic areas exhibited a high percentage of pediatric patients (20—30% of affected patients). Clinical manifestations of childhood brucellosis are varied and range from minimal symptoms to extreme morbidity and occasional fatality. Asymptomatic infections are also not uncommon. The fact that brucellosis is endemic in the Kingdom became clear in the early 1980s. Several reasons have been considered, but the most prominent of them is the increase in the importation of animals from areas where brucellosis is endemic, especially some African countries. Consumption of raw milk and, to a lesser extent, contact with infected animals or their products are the primary routes of infection. The consumption of fresh, unpasteurized milk from camels is a traditional practice, and people believe that boiling the milk removes nutritional value.

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1. Purpose of the guidelines

1. To increase the awareness of pediatricians and other child care providers, such as family medicine and general practitioners, about brucellosis, its varied clinical presentations and its impact on child health.
2. To provide insight to the epidemiology of brucellosis in Saudi Arabia, the modes of transmission and preventive measures.
3. To set a standard scheme for the diagnosis, antimicrobial therapy, management and follow up of childhood brucellosis.

2. Epidemiology

Brucellosis is endemic in Saudi Arabia with a reported incidence of 18/100,000 population/year, as reported by the MOH in 2011. An overall seroprevalence rate of 15% was found in the Saudi population, and the seroprevalence in children aged 0—14 years was 10% [1].

The reason for the high prevalence of brucellosis in Saudi Arabia is attributed, but not limited, to the following:

1. The nomadic life style including animal raising, especially of sheep and camels.
2. The traditional belief of the great benefit of ingesting raw milk, especially camel milk.
3. The high rate of animals imported from Africa where the disease is endemic, with a lag in compliance with national and international policies of animals screening and quarantine rules. In Saudi Arabia, the prevalence of *brucella* among different animals is high; 8% in camels, 18.7% in cattle, 6.5% in sheep and 9.7% in goats [2].
4. The mixing of different animal herds together, such as raising sheep and cattle together.
5. The low levels of public awareness about the seriousness of brucellosis as a human disease.
6. The resistance to slaughtering infected animals.

3. Transmission

Brucella spp. are small, gram negative, non-motile, non-spore-forming, rod shaped (coccobacilli) bacterial organisms. It is a zoonotic disease caused by the ingestion of raw unpasteurized milk from infected animals or close contact with their secretions. There are different animal reservoirs for different *Brucella* spp. that are known to cause human disease:

Species	Animal Host	Virulence
Brucella melitensis	Goats, sheep, camels	++++
Brucella abortus	Cows, other bovidae animals and camels	+++
Brucella canis	Dogs	+
Brucella suis	Pigs	+

Other species include *Brucella ovis*, *Brucella neotomae*, *Brucella microti* and marine *Brucella* species (*Brucella pinnipediae* and *Brucella cetaceae*); however, only the marine species have been reported to rarely cause human disease [3].

In Saudi Arabia and most of the neighboring countries, the most prevalent species is *Brucella melitensis* causing 70—90% of brucellosis cases. *Brucella abortus* is the second most common disease causing pathogen. The other species rarely cause brucellosis.

Transmission of the organism to humans occurs as a result of:

1. The consumption of unpasteurized raw milk or other dairy products, especially soft cheese, butter and cream. Hard cheese, sour milk and yogurt are unlikely to transmit the disease because of the propionic and lactic fermentation.
2. Direct contact with the secretions of infected animals or their products, such as the placenta or aborted materials.
3. The air borne transmission of aerosolized materials through open wounds or mucus membranes either in the animal areas or in the laboratory when dealing with blood and other infected fluid cultures. It has been found that direct contact with soil, animal feces and dust contaminated with *Brucella* is associated with a higher risk of infection [4].
4. Vertical transmission, sexual transmission and breast milk transmission are anecdotally reported routes of infection.

4. Clinical presentation

Most infected children have acute and sub-acute presentation of 2—4 weeks of symptomatology [5,6]. Brucellosis symptomatology is very variable, in part because of the variable pathogenicity of different strains. It is known that *B. abortus* causes milder disease with either mild symptomatology or focal lesions. However, infection with *B. melitensis* is usually associated with a high rate of bacteremia, short incubation periods, and noticeable symptoms [3,6,7]. Almost any organ can be affected and varied complications are reported. Almost 76% of affected patients present within two weeks of the onset of their symptoms [4]. Fever and arthritis are the most common presenting signs. Monoarthritis was more common than polyarthritis. This may create confusion with pyogenic arthritis in children; therefore, in a community where *brucella* is common, awareness about this entity should prompt the investigation of this disease, and physicians should have a high index of suspicion for *Brucella* arthritis. The most commonly affected joints were the hip and knee. Unlike in adults, the sacroiliac joint and the axial skeleton were rarely affected [4]. *Brucella* osteomyelitis is rare in children. Studies showed that only l—2% of children with brucellosis has osteomyelitis [5,6,8]. Neurobrucellosis is rare in children and it has been reported in only 0.5—1% of children with brucellosis. The clinical presentation of neurobrucellosis is variable with meningitis and

meningoencephalitis being the most common. Other presentations of neurobrucellosis include polyradiculopathy, myelitis and psychiatric disorders, mainly depression. Rare CNS manifestations include brain abscess, hydrocephalus, pesudotumor cerebri, Guillain Barre syndrome, cranial nerve palsy, cerebral venous sinus thrombosis, subdural and epidural collection and stroke [9,10,11]. Clinical manifestations of neurobrucellosis range from subtle manifestation, such as fever and malaise, to frank meningitis. Other manifestations include cranial nerve neuropathy, hearing loss, visual disturbances, chronic vomiting, altered sensorium and coma and various neurological focal motor deficits. Recovery is typical and sequelae are rare.

Classic general symptoms include the following:

- Headache
- Myalgia/bone pain
- Anorexia/weight loss
- Sweats
- Malodorous perspiration
- Depression, or mood disorders
- Fever
- Arthralgia/arthritis

The main signs are:

- Fever
- Arthritis
- Hepatomegaly
- Splenomegaly
- Neck stiffness
- Miscellaneous (skin rash, cervical nodes, drowsiness, periorbital swelling, ataxia)

Other rare manifestations include:

- CNS meningitis, encephalitis, meningoencephalitis, brain abscess, and Guillain Barr syndrome
- Lung pneumonia
- Cardiac endocarditis and myocarditis
- Liver transaminasemia and abscess
- Spleen abscess
- Genitourinary epedidemo-orchitis and nephritis
- Eye uveitis
- Thyroid abscess
- Spine epidural abscess
- Hematologic immune thrombocytopenic purpura and hemophagocytic syndrome

4.1. Diagnosis

Laboratory diagnosis of brucellosis relies on:

1. Positive blood or other sterile body fluid culture, such as synovial fluid, CSF, and plural fluid, for *brucella* species.
2. Or, a positive *brucella* serology test of 1:$\geq$160, using the Standard Agglutination Test (SAT) for patients presenting with symptoms suggestive of brucellosis. For the purpose of screening and in the absence of clinical indicators of active brucellosis, a titer of 1:320 or higher is more specific for the presence of the disease.

4.2. Culture

Although positive culture should be the gold standard for diagnosis, its yield remains suboptimal. The culture yield is greater in the first two weeks of illness and as the duration of illness increases the yield decreases. In a patient with fever and chills and short duration of illness, the culture yield is up to 90%; however, as the disease duration increases the yield drops to 30%. Most studies have shown a 40—70% yield. Rapid diagnosis of *brucella* using the new third generation continuous monitoring blood culture systems, such as non-radiometric BACTEC or BACT/ALERT, has shown a better and faster recovery with all positive cultures occurring by day 5—7 of incubation; therefore, extending the blood culture incubation to 4 weeks for improving the yield of culture is rarely required [12,13].

4.3. Specimen collection and requisition

Blood is collected aseptically by venipuncture. The volume withdrawn at a single venipuncture (3—5 ml and 10 ml from older children) and inoculated into a pediatric vial. For the diagnosis of bloodstream infection, two "sets" drawn 20 min apart are normally recommended. In infants and children, partly because the intensity of bacteremia is greater and partly due to physiological reasons, 3.0 ml in the specially formatted pediatric vial is acceptable. Inoculated vials should be transported promptly to the laboratory and held at room temperature until they are entered into the BacT—Alert system. Vials must be incubated up to 5 days routinely, in cases with a high index of suspicion along with a negative initial blood culture, the incubation period can be increased up to 21 days.

4.4. Species identification

- Gram stain: *Brucella* are Gram-negative coccobacilli that strongly retain the crystal violet and resist counterstaining. It may be necessary to counterstain for 1—3 min.
- For any blood culture that flags positive and is indicative of *Brucella* species, 0.5 ml of the blood culture fluid is to be aspirated from the bottle and inoculated onto a urea slant. This tube is sealed with parafilm and incubated in CO2 for 24 h. These slants must be checked for a positive reaction every 2 h and the length of time taken to become positive is documented on the electronic worksheet. *Brucella* is usually oxidase positive, catalase positive and urease positive.

4.5. *Brucella* susceptibility testing

Brucella susceptibility testing is performed by E-test for the following antibiotics: tigecycline, SXT, rifampin, tetracycline, streptomycin and ciprofloxacin. The interpretation is according to the CLSI guidelines as well as published references. Some discrepancy exists between in vitro susceptibility results and in vivo outcomes. This precludes the exact extrapolation of in vitro results to in vivo application. However, every attempt should be made to follow susceptibility data in the choice of antimicrobial regimen for the treatment of brucellosis [13,14,15].

5. Serology

Because of the lower yield of culture, serological tests remain the best diagnostic modality available. There are multiple serological tests for *brucella* including the standard agglutination test (SAT), the enzyme linked immunosorbent assay (ELISA), indirect Coombs, brucellacapt, indirect fluorescent antibody and immunochromatogrphic lateral flow [13,14]. The most commonly used tests are SAT and ELISA.

5.1. Standard agglutination test (SAT)

SAT is the most commonly used and most standardized test. It is based on measuring an agglutination titer of different serum dilutions against a standardized concentration of whole *brucella* cell suspension. It is usually measured by doubling the serum dilution from 1:20 up to 1:20,480. A positive titer is 1:160 or more. In endemic areas, there may be a persistent and continuing exposure to the source of infection, and therefore there may be a persistent low titer in the range of 1:80—1:160 in the absence of true infection. It was found that 2.9 and 2.5% of the healthy Saudi population have a titer of 1:160 and 1:320, respectively [1]. Our study showed that 92% of children with acute brucellosis have a titer of 1:320 or more [6]. *Brucella* antibodies can persist for a long period after acute infection with a median time to serological cure of 18 months. Among cured patients, 29% continued to have a titer of 1:320 or higher 2 years or more after infection [16,17]. Therefore, the interpretation of serology in endemic areas should be correlated to the clinical presentation. As such, for screening purposes and in the absence of suggestive symptomatology, a titer of 1:320 or more should be taken as the cutoff level for positivity. Coupling SAT with the 2-mercaptoethanol agglutination test is useful in differentiating acute from chronic brucellosis. The 2-mercaptoethanol agglutination test elutes IgM out leaving IgG. Elevated IgG titers indicate an acute disease. A negative 2-mercaptoethanol test after therapy indicates a good response to treatment.

There are a few limitations to the SAT including:

- The inability to diagnose *B. canis*
- The appearance of cross-reactions of IgM with *Francisella tularensis*, *Escherichia coli* O116 and O157, *Salmonella urbana*, *Yersinia enterocolitica* O: 9, *Vibrio cholerae*, *Xanthomonas maltophilia*, and *Afipia clevelandensis*
- The SAT may be negative in presence of disease in rare occasions

Lack of seroconversion can be attributed to:

- The performance of the SAT early in the course of infection
- The presence of blocking antibodies
- The prozone phenomenon (i.e. the inhibition of agglutination at low dilutions due to an excess of antibodies or to nonspecific serum factors)

5.2. Enzyme linked immunosorbent assay (ELISA)

The ELISA is performed using 96-well microtiter plates that are precoated with a standardized antigen, usually purified lipopolysaccharide. It has the advantage of measuring different classes of reactive antibodies including IgG, IgA and IgM. Thus, it has greater ability to differentiate between acute infection and relapsing infection. Various studies showed different results regarding the sensitivity of the ELISA. In general, the sensitivity ranges from 60 to 98%. In acute brucellosis, the sensitivity and specificity of ELISA for IgG were found to be 45 and 97%, respectively, and were 79 and 100% for IgM, respectively [18]. Another study found that the sensitivity and specificity of ELISA for IgG were 84 and 100%, respectively, and 60 and 100% for IgM, respectively [19]. The sensitivity and specificity of combined of ELISA for IgG and ELISA for IgM are comparable to that of the SAT [14]. Therefore, the SAT remained the preferred test of choice for acute brucellosis. However, for follow up and for the diagnosis of a *brucella* relapse ELISA is better as it gives a separate titer for IgG, IgM and IgA and thus can be used to assess response to therapy and relapse. The ELISA is the best for detecting *brucella* antibody in the cerebrospinal fluid in cases of neurobrucellosis [14].

5.3. PCR-based diagnosis

Various PCR tests are being studied for diagnosing brucellosis both at the genus and species levels. These tests have shown promising results regarding their sensitivity, specificity and clinical applicability. They have advantages over the gold standard of culture isolation in that they are easy to perform, require a short period of time and avoid the risk of laboratory acquired infection. Once validated, these diagnostic tests will be the future diagnostic modality of choice [20,21].

6. Laboratory investigation

All patients suspected of having *brucella* should have the following tests done:

1. Complete blood count and differentiation:
   The result will be normal in most cases; however, in some patients variable affection of different cell lines may be noted such as leukopenia, anemia, thrombocytopenia or a combination of some or all of them.
2. Erythrocyte sedimentation rate:
   As an acute reactant marker, it will be raised but usually of modest value ranging from 20 to 80 mm/h.
3. Liver function test:
   A mild to moderate elevation of transaminases can be found in some cases. Increased bilirubin is rare but it can occur.
4. Renal function test:
   Mostly normal; however, in rare cases, glomerulonephritis may occur with variable elevations of creatinine and BUN.
5. Blood culture

Table 1 Drugs used for brucellosis and their dosages.

Drug	Dosage
Rifampicin	20 mg/kg/day in two divided doses (max. 600 mg)
Doxycycline	5 mg/kg/day in two divided doses (max. 200 mg) (only for children more than 8 year of age)
TMP/SMX	10 mg of trimethoprim/kg/day (max. 480 mg)
Gentamicin	5–7.5 mg/kg/day IM or IV either as a single dose or three divided doses
Streptomycin	15 mg/kg IM or IV once daily (max. 1 g/day) (only for children more than 8 year of age)
Ciprofloxacin	30 mg/kg/day in two divided doses (max. 1.5 g)

6. Other sterile body fluid, tissue or bone marrow culture as indicated.
7. *Brucella* serology

7. Management[4,9,10,11,21,22,23]

Management of brucellosis relies on adherence to the following criteria:

1. Using an antibiotic that has the ability to act intracellularly and in acidic media.
2. Using combined therapy.
3. Using antimicrobials for a prolonged duration according to the system involved.

There is a limited number of antibiotics that can be used for the treatment of *brucella* infections including doxycycline, rifampicin, trimethoprime–sulfamethoxazole, streptomycin, gentamicin and ciprofloxacin (see Table 1).

- A common combination for children yielding successful results is as follows:
  - Rifampicin and TMP/SMX for children below 8 years of age.
  - Doxycycline and TMP/SMX or rifampicin for children older than 8 years of age. This combination has been shown to have the highest success rate and should be used in children above 8 years to avoid the staining of the teeth in younger children.
  - In serious infections, such as neurobrucellosis and endocarditis, three to five drugs need to be used for a longer period of time, usually for three to 12 months (see Table 2).
  - Gentamicin for 7 days or streptomycin for 14 days can be used for patients requiring hospitalization.
  - The use of streptomycin has been associated with a lesser degree of relapse but is not significantly superior.

8. Monitoring the response to therapy

8.1. Clinical response

Most patients respond promptly to therapy. Additionally, most patients with acute brucellosis without neurobrucellosis or *brucella* endocarditis can be managed as

Table 2 Regimes for different manifestations of brucellosis.

Disease	Therapy		Comment and duration of therapy
	Children < 8 years	Children > 8 year	
Common diseases: Acute brucellosis, *brucella* arthritis, *brucella* osteomyelitis, *brucella* bacteremia	Rifampicin and septra OR rifampicin for 45 days and gentamicin for 7 days	Doxycycline and rifampicin OR Doxycycline for 45 days and streptomycin for 14 days OR Doxycycline for 45 days and gentamicin for 7 days	Hospitalized patients add gentamicin for 5–7days Duration of therapy 6 weeks
Serious illness *Brucella* endocarditis	Rifampicin, septra, and ciprofloxacin	Doxycycline, septra, and rifampicin	Gentamicin for the initial two weeks Surgical intervention is indicated Duration of therapy is 3–9 months
Neurobrucellosis	Rifampicin, septra, and ciprofloxacin	Doxycycline, septra, and rifampicin	Gentamicin for the initial two weeks Ceftriaxone has shown some efficacy and it is usually used in the initial therapy for 2–4 weeks Duration of therapy is 3–6 months up to one year in complicated cases

outpatients even if they are bacteremic. They usually show improvement within 3—7 days after starting therapy. All patients who are started on therapy for brucellosis should be followed closely in the clinic to monitor the persistence of the response and compliance to therapy. In patients who have CBC laboratory abnormalities, such as a positive blood culture and/or liver enzymes, they should have their tests repeated one week after starting therapy. Such patients usually normalize their laboratory abnormalities by then. If culture yields a positive result, attention needs to be paid to the susceptibility pattern although most *brucella* isolates remain sensitive to the first line antibiotics.

8.2. Serology response

Brucella titers decline slowly and may remain moderately high for months. Therefore, there is no need to repeat a titer early in the course of therapy. One serology titer should be repeated by the end of therapy to evaluate the trend and demonstrate a decrease in the titer. However, even if the titer remains high or decrease only slightly this does not mean that the patient did not respond as some patients may maintain high titers for a prolonged period of time. Almuneef et al. demonstrated that a titer over 320 can be sustained for up to 18 months in 25% of patients after resolution of the infection [16,17].

8.3. Relapse

Among treated patients, 3—9% will have a relapse or reinfection. Most relapses occur in the first year following therapy. If an affected patient begins to have symptoms, serology and blood culture should be repeated. Sites that may be affected such as the CNS or heart should be examined fully. The titer usually will be raised. However, obtaining an estimation of the IgG and IgM levels separately is optimal. This can be obtained by the ELISA or SAT with 2-ME. If the IgG level is high, relapse is indicated. Once relapse or reinfection occurs, referral to a pediatric infectious disease clinic is warranted for diagnosis confirmation and further management.

8.4. Prevention

1. Increase public awareness about the endemicity of brucellosis in Saudi Arabia and the importance of avoiding all risk factors that could lead to acquiring this infection. This entails stressing the importance of avoiding drinking raw milk or its products, and avoiding contact with sick animals or their products such as aborted fetuses.
2. Animal owners should be aware that brucellosis is prevalent among animals and thus regular checkups of animals are required.
3. Avoid mixing different herds of animals together as this practice facilitates the transmission of disease among animals.
4. The government should stress the screening of animals, the vaccination of seronegative animals and slaughtering diseased ones.
5. A collaborative team to implement a *brucella* 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.
6. Screening the family members of patients with acute brucellosis in endemic areas is strongly recommended to enhance the detection rate, to initiate early treatment and to reduce complications.

Where resources are limited, the screening of family members could be limited to the symptomatic and to children [24]. In these cases, the cutoff limit for a positive titer is 1:320.

Conflict of interest

There is no conflict of interest.

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