Pyomyositis of Infraspinatus and Supraspinatus Muscles: An Unusual Presentation of Brucellosis with Prozone Effect

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Abstract

Brucellosis is a bacterial zoonotic infection acquired by the consumption of dairy products or contact with infected animals' tissues or fluids. Here we report a case of a women who presented with a prolonged fever, night sweats, weight loss and joints pain. She was found to have shoulder pyomyositis and spondylodiscitis. Her initial *Brucella* serology showed a titer of 1:80 for *Brucella abortus*. The patient reported consuming homemade cheese made from unpasteurized cow's milk. A repeat *Brucella* serology performed with further dilutions showed *Brucella abortus* titer of 1:160 and *Brucella melitensis* titer of 1:5120.

This case report presents focal pyomyositis of the shoulder muscles as one manifestation of brucellosis. It also illustrates an important microbiological phenomenon of falsely negative *Brucella melitensis* titer due to prozone effect which highlights the importance of diluting the sample.

Keywords: Brucellosis; Pyomyositis; Prozone Effect.

Introduction

Brucellosis is a bacterial zoonotic disease caused by *Brucella* species; fastidious, aerobic, small, Gram-negative coccobacilli. The majority of human infections are caused by four species: *Brucella melitensis* (goats, sheep, and camels), *Brucella abortus* (cattle), *Brucella suis* (pigs), and *Brucella canis* (dogs). *Brucella* is transmitted to humans by consumption of dairy products, direct contact with infected animals' tissues or fluids, or inhalation of aerosols. Febrile illness with or without musculoskeletal involvement is the most common manifestation. Less common presentations include endocarditis, neurobrucellosis and rarely, hemophagocytic lymphohistiocytosis.[1,2] Diagnosis of brucellosis can be challenging due to variable and nonspecific clinical presentation and self-limiting nature of some of the symptoms in a small group of patients.[3,4]

Brucellosis can be diagnosed in the laboratory using conventional culture method, serology and nucleic acid amplification tests, e.g., polymerase chain reaction (PCR).[5,6] Brucella species are slow-growing, typically

requiring 2-3 days for detectable growth. Serology testing is another commonly used diagnostic modality, e.g., standard tube agglutination test (SAT) and enzyme-linked immunosorbent assays (ELISA).[5,7]

There are several challenges associated with the diagnostic tests currently available for brucellosis. For example, *Brucella* species on culture is a level 3 pathogen which requires special enhanced biosafety measures to prevent transmission to the laboratory personnel. These measures are not widely used in the laboratories.[8,9] In addition, there are some limitations of the available serology tests. One example is the "prozone" phenomenon seen with SAT and results from the inhibition of agglutination at low dilutions due to excess antibodies, thus giving falsely negative serology results. This highlights the need for preparing higher serum dilutions in cases with negative serology if brucellosis is suspected.[10,11]

This case report demonstrates an unusual presentation of brucellosis with shoulder pyomyositis . It also highlights the impact of prozone effect in the serological diagnosis of brucellosis which may make the diagnosis challenging.

Case Report

A 55-year-old Omani woman, presented to the emergency department at Sultan Qaboos University Hospital with an 8-month history of intermittent low-grade fever, right shoulder pain, back pain, and significant weight loss. She has hypertension, diabetes mellitus and dyslipidemia. Patient was seen in different health care institutions locally and abroad and no diagnosis was made. Upon further questioning, the patient gave a 9-month history of travel to Salalah, which is known for high rate of *Brucella* infection, but she denied consumption of unpasteurized dairy products or contact with animals while there. Three to four weeks after her return from travel she started having fever, night sweet, weight loss, along with right knee pain followed by back pain and subsequently right shoulder pain without swelling or redness. She lost around 13 kilograms since the onset of symptoms.

Upon presentation to our hospitals, her fever and constitutional symptoms had already improved but she had persistent right shoulder and knee pain with limited joints mobility. The rest of systemic review was unremarkable.

On clinical examination, she looked well, afebrile with normal vital signs. There was around one cm-palpable left cervical lymph node. There were no palpable lymph nodes in other areas or hepatosplenomegaly. Examination of the right shoulder and right knee did not show any redness, tenderness, warmth or effusion clinically. Other systems examination was normal.

Laboratory investigation revealed anemia with hemoglobin level of 9 g/dL otherwise inflammatory markers, renal profile, liver profile, bone profile were all normal. Multiple myeloma work-up was negative. Ultrasound of the right shoulder and the knee was normal. Blood culture, HIV serology and other relevant investigation were negative (Table 1).

Table 1: laboratory and microbiological tests results.

Test (reference range)	Value	Test (reference range)	Value	Microbiology
Hemoglobin (11.5-15.5 g/dL)	11.5 g/dL	Calcium (2.15-2.55 mmol/L)	2.59	Blood culture: negative
Platelets (150-400 x 10^9/L)	385 10*9/L	Phosphate (0.81-1.45 mmol/L)	1.21	CMV PCR: negative
White blood cells (4.5-11.0 x 10^9/L)	10.1 10*9/L	Magnesium (0.66 -1.07 mmol/L)	0.76	EBV PCR: negative
Absolute neutrophilic counts (1.8-7.7 x 10^9/L)	6.4 10*9/L	Urine protein electrophoresis: UPEP	No free light chain Monoclonal band detected	TB gene Xpert: not detected

C-reactive protein (<5 mg/L)	10 mg/L	Serum protein electrophoresis	high IgG = 28.5 g/L: normal 7-16)	*Coxiella burnettii* serology: negative
Creatinine (53-97 µmol/L)	33 umol/L,	ANA, ENA, anti-CCP	negative	
eGFR (>90 mL/min/1.73m^2)	>90	Free T4 pmol/L	19.7	Urine culture: negative
HCO3 (22-29 mmol/L)	24	TSH mIU/L	2.13	CA 19-9 = 4
Urea (2.5-7.1 mmol/L)	5.6	ALT U/L	18	CA125 = 9
Sodium (135-145 mmol/L)	135	AST U/L	15	CA 15-3 = 9.9
Potassium (3.5-5.1 mmol/L)	4.3	ALP U/L	106	AFP <2
Bilirubin	5	Total protein g/L	91	CEA ug/L = 1.1
Calcium (2.15-2.55 mmol/L)	2.21	albumin (35-45 g/L)	42	LDH U/L = 102

ANA: antinuclear antibodies, ENA: extranuclear antibodies, anti-CCP: anti cyclic citrullinated peptides antibody, LDH; lactate dehydrogenase.

Her initial *Brucella* serology showed a titer of 1:80 for *Brucella abortus* and non-reactive serology for *Brucella melitensis*. This result has triggered further questioning of the patient of other epidemiological factors for Brucellosis which turned to be positive for ingestion of homemade cheese that is produced locally from fresh cow milk. A repeat *Brucella* serology performed with further dilutions showed *Brucella abortus* titer of 1:160 and *Brucella melitensis* titer of 1:5120.

A Computed tomography (Pan-CT) of neck-chest-abdomen-pelvis with contrast showed right shoulder infraspinatus and supraspinatus muscle collection representing muscular abscess, measuring about 11 cm in craniocaudal dimension and 2 cm in transverse dimension [Figure 1]. There was also significant adenoid and tonsillar enlargement, a few reactive cervical and axillary lymph nodes and multiple vertebral sclerosis with features of spondylodiscitis. Right lower limb CT with contrast showed no collection or signs of inflammation. An aspirate of the collection in the infraspinatus & supraspinatus muscles was sent for culture. It grew Gram-negative coccobacilli which was later identified by PCR as *Brucella* species. Transthoracic echocardiogram showed no vegetation. PET-CT showed no other disease activity outside the above-described body areas. After consultation with infectious diseases team, patient was started on intravenous gentamicin 5 mg/kg daily for seven days in combination with oral doxycycline 100 mg every 12 hours and oral rifampicin 600 mg daily for complicated brucellosis. Patient received 5 months course of oral doxycycline and rifampicin with complete clinical and radiological response.

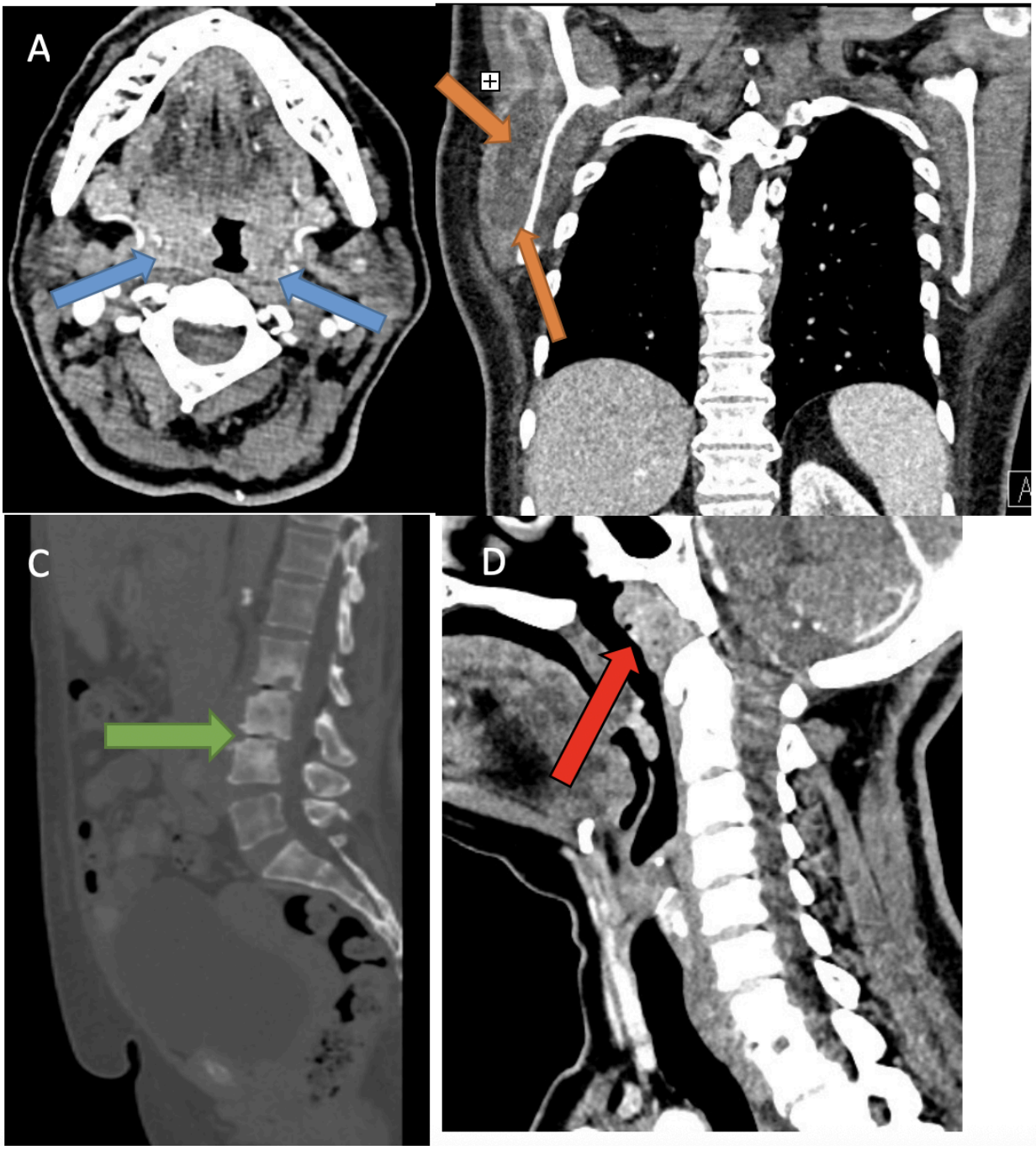

Figure 1: (a) Axial CT image show enlarged both tonsils (marked with blue arrow) but no definite evidence of low density to suggest any collection. **(b)** Coronal reformatted image of the chest show, that there is a collection noted in right supra and infraspinatus muscle region which is showing central low density and is showing peripheral enhancement (marked with orange arrow). **(c)** Sagittal CT reformation of lumbar spine show multilevel bony sclerosis involving L2, L3 and L4 vertebral bodies with bony sclerosis and reduced intervertebral disc space heights and end plate changes (marked with green arrow). Features are more in favoring of spondylo-discitis. **(d)** Sagittal CT reformation of the neck show enlarged adenoids with mild narrowing of nasopharyngeal airway (marked with red arrow)

Discussion

Osteoarticular involvement is the most common complication of brucellosis with sacroiliac joint and spine being the most affected sites. Involvement of the shoulder and particularly pyomyositis of shoulder muscles is uncommon presentation. Hassan KS et al. reported peripheral joints involvement in 15.9 % of patients with brucellosis.[12]

One study from Oman showed positive *Brucella* serology in 93.7 % of cases with brucellosis.[12] Blood culture is positive for *Brucella* species in 6-40% and 70-90 % according to two studies respectively.[13,14]

Molecular testing for brucellosis, e.g., PCR, is highly sensitive with short turn-around time compared to culture, but it is not widely available. However, the performance of PCR assays may vary between laboratories.[15] On the other hand, *Brucella* serology is more widely used with *Brucella* IgM antibodies appear within one week of infection and remain elevated for months. IgG antibodies appear within 3 weeks of infection and remain at low levels for months to years after clinical recovery.[16] Examples for serological assays include 2-mercaptoethanol (2-ME), standard tube agglutination test (SAT) and enzyme linked immunosorbent assay (ELISA).[17]

The detection of antibodies directed to the O-chain component of the lipopolysaccharide (LPS) antigen, expressed at the surface in brucella species with smooth phenotype in agglutination tests is the main method used for diagnosis of brucellosis but due to prozone phenomenon, other tests may be needed.[18,19]

In our patient initial sample with the falsely negative result was repeated twice by two different individuals as per the package insert (i.e., diluted up to 1:1280), and both got negative results. This is most likely due to prozone phenomenon (excess antibodies) which is not commonly seen at this dilution. Patients with prozone effect may require sample dilution up to 1:2560 for it to show positive as reported in previous literature.[11] An old study suggested that this phenomenon is triggered by the temperature at which agglutination test is done.[20] Although this was not confirmed in further studies. The more obvious reason for this phenomenon, which is seen in other serology testing (e.g. syphilis), is the excess of antibodies that leads to inhibition of agglutination at low serum dilutions. There are other factors that might contribute to this phenomenon including the broad and unpredictable variability of the host's immune response to *Brucella* antigens as well as the variation in the quality of *Brucella* antigens. These factors must be taken into consideration especially in endemic areas**.**[21,22]

Our case highlights a unique clinical presentation with pyomyositis of shoulder muscles in addition to right knee and spine. Further history of exposure in our case was triggered by the initial reactive *B. abortus* serology, despite the negative *B. melitensis*. This identified an unexpected source of brucellosis of homemade cheese that is only consumed in specific regions in Oman which makes exposure history incomplete by standard history questions. This brings into attention the third unique element of this case, which is the consideration of prozone effect and the need for sample dilution if brucellosis is highly suspected.

Conclusion

Brucella infection varies in presentation and sources of infection. A high clinical suspicion and consideration of rare possible sources (local made cheese) and rare presentations (shoulder pyomyositis) are very important. Consideration of false negative testing due to prozone effect with agglutination test is important in cases of high suspicion of *Brucella* infection for which samples dilution will be needed.

Disclosure

There is no conflict of interest to declare. A verbal consent taken from the patient for case writing and publication.

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