Diagnostic challenges of human brucellosis in Hong Kong: a case series in two regional hospitals

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A retrospective analysis of six patients diagnosed with brucellosis in two regional hospitals was carried out. The epidemiological, clinical, and laboratory features were studied. All patients had exposure history. Three patients presented with musculoskeletal symptoms, while three had predominantly genitourinary symptoms. One patient did not have fever at presentation. All patients were diagnosed by positive blood culture of *Brucella melitensis*, and the diagnosis was not suspected for all except one patient at presentation. Given the inferior sensitivity of blood culture to serology, human brucellosis may be underdiagnosed, especially when the index of suspicion is low.

Introduction

Human brucellosis is a chronic granulomatous zoonosis caused by the facultative intracellular bacteria of the genus *Brucella*. It is endemic in the Mediterranean region, including Turkey, the Arabian peninsula, the Indian subcontinent, Mexico, and parts of Central and South America.[1] Among the species, *Brucella melitensis* (goats, sheep) and *Brucella abortus* (cattle) are the most virulent in humans. Infection can occur through consumption of infected unpasteurised dairy products and raw meat. Other routes of transmission include contamination of skin abrasions, inhalation of airborne aerosols from animal manure or bacteria culture and, rarely, person-to-person transmission.[2] The clinical presentations are protean and can be non-specific, presenting diagnostic challenges in non-endemic areas such as Hong Kong. This report describes a case series of human brucellosis in two regional hospitals.

Case series

A retrospective analysis of six patients with brucellosis from January 2006 to December 2009 in Princess Margaret Hospital and Yan Chai Hospital, Hong Kong was carried out. The diagnosis of brucellosis was made by isolating *Brucella* species from blood (BACT ALERT; bioMérieux sa, Marcy l'Etoile, France). All isolated strains were sent to the Public Health Laboratory Centre, Department of Health, Hong Kong, for confirmation and biotyping. In addition, patients' sera were tested for *Brucella* antibody by the standard tube agglutination test (Remel, Inc, Dartford, UK).

The mean age of the patients was 48 years (range, 26-56 years). There were four men and two women (Table 1). The main clinical presentations were fever (n=5) and low back pain (n=3). Most patients had normal white blood cell counts with relative lymphocytosis and mild anaemia, and elevated C-reactive protein (Table 2). The patients' liver function tests were mildly deranged and two patients had mild hepatomegaly (Table 3). Blood culture revealed small Gram-negative coccobacilli identified as *B melitensis*. All patients were treated with doxycycline and either streptomycin or rifampicin with good effect (Table 4).

Key words
Anti-bacterial agents; *Brucella melitensis*; Brucellosis

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Discussion

Brucellosis has been included in the list of infectious diseases of public health concern since 2004. Up to 2007, seven cases of human brucellosis were reported in Hong Kong.[3] In this case series, six patients were diagnosed with brucellosis by positive blood culture in two regional hospitals from 2006 to 2009. All patients were infected with *B melitensis* and they all had relevant exposure or occupational histories. Patient 1 was a cook and patient 2 was a butcher. Although they did not have a history of contact with goats or sheep, they might have acquired the infection through minor skin abrasions when handling raw beef. This route of transmission is not totally unexpected as *B melitensis* infection in cattle has been reported in Israel, Kuwait, and Saudi Arabia.[4]

具診斷挑戰性的人類布氏桿菌病：香港兩間分區醫院的病例系列報告

本文回顧分析香港兩間分區醫院中的6名布氏桿菌病患者，包括其發病、臨床及實驗數據的特性。所有患者有病菌接觸史，並對*Brucella melitensis*血液培養呈陽性反應。當中1人病發時並無發燒，3人有肌肉骨骼性徵狀，另3人的病徵集中在生殖泌尿系統上。5名病人病發時均未能診斷布氏桿菌病。由於人類布氏桿菌對血清培養的敏感度較低，在警覺性也較低時或未能即時診斷人類布氏桿菌病。

Brucellosis was suspected at presentation for only one patient (patient 3) who was given a presumptive diagnosis of fever of unknown origin, and was tested for *Brucella* antibody before the positive blood culture result was received. The difficulty in diagnosing brucellosis might be due to several reasons. First, the exposure history might be distant from the time of presentation, as the incubation period ranges from 2 to 5 months, and patients might not volunteer this information unless specifically asked. Second, brucellosis can be sexually transmitted,[2] and patients 3 and 4 were married to each other. Sexual transmission could not be confirmed for these patients as they had the same exposure history and no genital specimens were saved for culture. Nevertheless, it is worthwhile exploring a spouse's exposure history based on clinical suspicion. Third, the clinical presentations of human brucellosis can be protean and non-specific. In endemic areas, musculoskeletal involvement accounted for more than 40% of the cases,[5,6] while the genitourinary system was the second most common site of involvement.[6] This was also true of this series, with two patients being admitted to orthopaedic wards. The most common symptom among these patients was subacute low back pain, which can be easily overlooked. In particular, one patient (patient 2) did not have a fever at presentation, and was misdiagnosed with degenerative spondylolisthesis at the first admission. It was until computed tomography and magnetic resonance imaging of the spine showed infective spondylitis and blood culture was taken that he was diagnosed with brucellosis. Of note, fever was absent in up to 34% of patients in a series of *Brucella* spondylitis.[7] Therefore, brucellosis should be considered a possible diagnosis for patients with subacute low back pain, especially for those with an exposure history. Patients 5 and 6 came from Pakistan, where brucellosis is endemic.[8,9] With compatible clinical features, the diagnosis should not be dismissed in patients coming from endemic areas. Finally, patients 3, 4, and 6 presented primarily with genitourinary symptoms. Most patients with these symptoms would be discharged with empirical antibiotics without blood being taken, stressing the importance of enquiry into exposure history or ethnic origin of any patients presenting with symptoms of infectious diseases.

Laboratory findings can frequently help to support the diagnosis of brucellosis.[6] The findings for most patients of normal white blood cell counts with relative lymphocytosis and mild anaemia suggested chronic infection rather than a degenerative condition. In addition, liver function tests commonly showed a mild degree of derangement and two patients had mild hepatomegaly by ultrasound examination; this is largely accounted for by the tropism of *Brucella* species for the reticuloendothelial system. Lastly, C-reactive protein was more consistently elevated than erythrocyte sedimentation rate, which may also help differentiate brucellosis from other chronic infections such as tuberculosis.

The definitive diagnosis of brucellosis relies on isolating the bacteria from blood or tissue specimens. Automated blood culture systems used in most diagnostic microbiology laboratories can isolate 95% of *Brucella* species within 7 days.[10] In this series, all isolates were recovered within 4 days. On

TABLE 1. Clinical features of six patients with brucellosis

Patient No.	Sex/age (years)	Occupation	Medical history	Exposure history	Incubation (months)
1	M/52	Cook	Diabetes mellitus, hypertension, alcoholic cirrhosis	Prepared roasted meat in restaurant	Unknown
2	M/52	Butcher	Good	Handled raw meat	Unknown
3	M/56	Sewage plant worker	Good	Visited a local farm, husband of patient 4	3
4	F/54	Health care assistant	Good	Visited a local farm, wife of patient 3	4
5	F/46	Housewife	Good	Visited Pakistan, with goats in neighbourhood	5
6	M/26	Unemployed	Good	Consumed homemade yogurt in Pakistan	2

TABLE 2. Laboratory findings

	White blood cell count (x10^9/L)	Lymphocytes (%)	Haemoglobin (g/L)	Alanine aminotransferase (U/L)	Alkaline phosphatase (U/L)	C-reactive protein (mg/L)	Erythrocyte sedimentation rate (mm/h)	Rheumatoid factor
Reference range/level	4.5-11.0	34	120-175	10-40	50-120	<6.0	0-20	-
Patient No.								
1	7.1	20.9	93	33	178	28.5	91	Not done
2	6.7	38.8	115	46	116	35.6	70	Not done
3	4.2	39.9	128	49	137	12.1	10	Negative
4	4.2	56.0	113	85	84	60.5	26	Negative
5	6.8	47.2	117	20	74	52.5	93	Negative
6	11.9	9.1	139	57	78	111	47	Not done

Gram stain, they appeared as small Gram-negative coccobacilli. Rapid urease production (<30 minutes) can further support the identity of the isolates.[11] Additional evidence was the positive agglutination of the blood culture broth fluid with *B melitensis* monospecific antisera (Remel) and positive modified Ziehl-Neelsen stain.[4] All *Brucella* species are aerobic and oxidase-positive. Nevertheless, further identification of suspected isolates should only be performed in the reference laboratory using biosafety level 3 facilities, since laboratory workers are at risk for infection.[12] The identification is now commonly performed by molecular methods, such as 16S rRNA gene sequencing or polymerase chain reaction with random amplification of polymorphic DNA.[6]

Apart from culture, serology is also considered to be diagnostic in the context of compatible clinical features. Antibodies usually appear in the blood at the end of the first week of illness. The most popular test is standard tube agglutination, in which diluted serum is mixed with stained killed *Brucella* bacteria. The total quantity of antibodies (immunoglobulin [Ig] M and IgG) is measured. Titres of ≥160 are considered positive, although using 320 as cut-off maybe more specific in endemic areas.[6] Seroconversion and evolution of the titres over time are also indicative of brucellosis. In this series, all patients had *Brucella* antibody titres of ≥160, four of whom had *B abortus* antibody titres 2-fold greater than those for *B melitensis*. This result is not unexpected as serologic differentiation of infecting species is considered unreliable because of cross-reactivity.[13] Moreover, the test is not suitable for monitoring treatment response as titres can remain high for a prolonged period.[4] One patient (patient 1) had an *B abortus* antibody titre of 160 one year after presentation. When serology is used as the gold standard, the sensitivity of blood culture varies from 53.4 to 90.0%,[7] with decreasing yields over time with prior antibiotic therapy. Although bone marrow biopsy culture, lysis centrifugation technique, and blind subcultures for at least 4 weeks can improve the sensitivity of recovering the organism, positive *Brucella* serology may still be the only diagnostic clue for a considerable number of patients. In contrast, seronegativity is rarely reported.[14,15] Therefore,

Duration of symptoms (weeks)	Clinical presentations	Peak temperature (ºC)	Physical signs	Provisional diagnosis
12	Fever, low back pain, bilateral lower limb swelling with cellulitis	39.1	Reduced power of hip flexion and extension on right side	Degenerative spondylolisthesis
5	Low back pain, chills and rigor 3 weeks before back pain	37.0	Tenderness over lower thoracic region	Tuberculosis of spine
4	Fever, night sweats, headache, malaise urinary symptoms	38.2	Nil	Pyrexia of unknown origin
2	Fever, headache, malaise, bone pain, urinary symptoms	39.0	Nil	Urinary tract infection
4	Low back pain with fever for 2 days	39.5	Tenderness over lumbosacral region, lower limb power 4/5	Infective sacroiliitis
1	Right scrotal pain with fever for 2 days	40.5	Tenderness over right testis and epididymis	Epididymo-orchitis

TABLE 3. Radiological findings and microbiological findings

Patient No.	Relevant radiological findings*	*Brucella* serology at presentation and follow-up (titres)	Blood culture species	Incubation period (days)
1	Ultrasound abdomen: mild hepatomegaly, liver parenchymal diseases Radiography lumbar spine: L3/4 disc space narrowing Bone scan: increased uptake at L4/5, likely septic spondylitis and right sacroiliac joint septic arthritis	*B abortus* 640, 160 *B melitensis* 640, 80 (1 year apart)	*B melitensis* biotype 1	4
2	Radiography lumbosacral spine: normal CT/MRI spine: reduced T9/10 disc space with T9 and T10 vertebrae destruction, suggestive of tuberculosis infection Bone scan: active bone lesion at lower thoracic spine (T9/10), compatible with infective spondylitis/discitis at T9/10 level	*B abortus* 320, 160 *B melitensis* 160, 80 (2 weeks apart)	*B melitensis* biotype 1	4
3	Ultrasound abdomen: liver congestion	*B abortus* 1280 *B melitensis* 640	*B melitensis*	4
4	Ultrasound abdomen: normal CT brain: normal Bone scan: normal	*B abortus* 1280 *B melitensis* 320	*B melitensis*	4
5	Radiography lumbosacral spine: narrowing of right sacroiliac joint space (Fig) CT spine: suspected osteitis condensans ilii, increased right iliacus muscle thickness MRI spine: unilateral right sacroiliitis, likely infective	*B abortus* 320 *B melitensis* 80	*B melitensis*	3
6	Ultrasound testes: right epididymo-orchitis	*B abortus* 1280, 1280 *B melitensis* 1280, 640 (1 week apart)	*B melitensis*	3

* CT denotes computed tomography, and MRI magnetic resonance imaging

TABLE 4. Antibiotic regimens and outcomes

Patient No.	Antibiotic regimen before diagnosis	Antibiotic regimen after diagnosis	Outcome
1	Ampicillin + cloxacillin for 3 days	Doxycycline + streptomycin for 2 weeks, then doxycycline for 10 weeks	Cured
2	Nil	Doxycycline + streptomycin for 3 weeks, then doxycycline for 3 weeks	Cured
3	Nil	Doxycycline + rifampicin for 3 weeks, then doxycycline for 3 weeks	Cured
4	Augmentin for 4 days	Doxycycline + streptomycin for 3 weeks, then doxycycline for 3 weeks	Cured
5	Augmentin for 8 days	Doxycycline + streptomycin for 3 weeks, then doxycycline for 3 weeks	Cured
6	Ciprofloxacin for 5 days	Doxycycline + streptomycin for 2 weeks, then doxycycline for 4 weeks	Cured

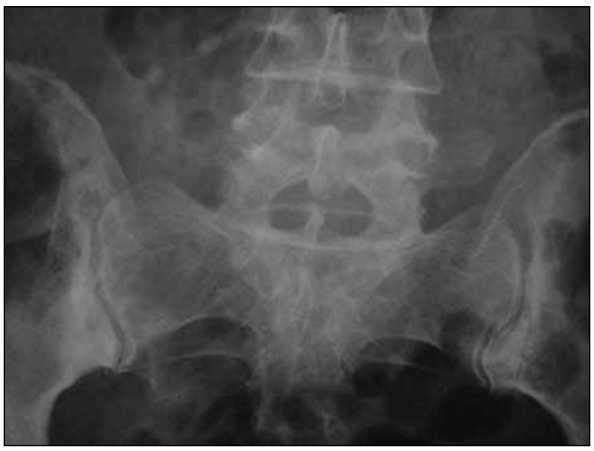

FIG. Radiograph of the lumbar sacral spine of patient No. 5

serology may be useful as a screening test in patients with suggestive clinical features.

Treatment of brucellosis is straightforward once the diagnosis is made. Common regimens include doxycycline for 6 weeks in combination with either rifampicin or streptomycin for the first 2 to 3 weeks, the latter being more effective for preventing relapse. Since treatment failure can be as high as 26% and the relapse rate can vary from 4 to 55%,[7] some clinicians advocate that treatment of *Brucella* spondylitis is extended to at least 3 months.[16]

Conclusion

Brucellosis poses significant diagnostic challenges to clinicians, due to its non-specific presentations

and the low clinical suspicion of clinicians. Exposure history is important as it can alert clinicians to the possibility of brucellosis. Additional clues include normal white cell count, relative lymphocytosis, mild anaemia, and elevated C-reactive protein and liver enzymes. In patients with features suggestive of chronic inflammatory processes, in particular, spondylitis or sacroiliitis, or in patients with epididymo-orchitis coming from endemic areas, brucellosis should be included in the list of differential diagnoses, even in the absence of fever. Serum should be sent for *Brucella* antibody and blood sent for culture. All patients in this series were diagnosed with brucellosis by positive blood culture. Given the fact that blood culture is only fairly sensitive, many cases may be undiagnosed, resulting in unnecessary morbidity and complications.

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