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A case of human brucellosis in Hong Kong

香港人類感染布氏桿菌病個案

Brucellosis is an infectious disease of humans and animals caused by *Brucella* species. We report on a 34-year-old housewife who presented with recurrent headache, fever, and malaise. Blood cultures yielded slow-growing gram-negative coccobacilli that were later identified as *Brucella melitensis*. The patient recalled handling goat placenta in China. She was prescribed a 6-week course of doxycycline and rifampicin. Laboratory staff who had been exposed to the isolate remained asymptomatic. The epidemiology, diagnosis, and treatment of brucellosis are discussed.

布氏桿菌病是一種由布氏桿菌屬引致的傳染病，人畜均有機會受到感染。本文報告一名34歲家庭主婦出現復發性頭痛、發燒和不適等徵狀。血液培養顯示其體內革蘭氏陰性球桿菌數目在緩速增加，稍後確定為馬爾他布氏桿菌。患者表示曾於內地處理羊胎盤，治療方面則須服食6星期的多四環素和利福平，而曾經接觸分離菌的實驗室人員並無發病徵狀。本文亦從流行病學、診斷和治療方法探討布氏桿菌。

Introduction

Human brucellosis is a multisystem and potentially lethal disease of zoonotic origin with highly variable and non-specific clinical presentation. It has a worldwide distribution and remains endemic in the Mediterranean basin, Middle East, western Asia, Africa, and Latin America.[1] The true incidence is unknown and varies widely, from lower than 0.01 to higher than 200 per 100 000 population.[2] We report a rare case of human brucellosis in Hong Kong.

Case report

A 34-year-old housewife with good past health presented with a 2-week history of headache, malaise, and two episodes of vomiting. She was born in Hong Kong and had travelled to Zhuhai 1 week prior to admission. In Zhuhai, she had remained in the city and reported no animal contact, insect bites, or consumption of raw food or unpasteurised dairy products. Other family members were asymptomatic. On admission, she was alert, febrile (38.6°C), and appeared non-toxic. Other vital signs were normal. There was no rash, no palpable lymph node, and no organomegaly. Physical examination was unremarkable.

Admission haematology showed a haemoglobin level of 116 g/L, leukocyte count of 6.1 x 10^9 /L, and platelet count of 270 x 10^9 /L. Erythrocyte sedimentation rate was 46 mm/h. Renal function was within normal limits but liver function was mildly deranged (alkaline phosphatase, 164 IU/L; alanine transferase, 113 IU/L; bilirubin, 7 μmol/L; gamma glutamic transpeptidase, 196 IU/L). Screening for hepatitis markers

Key words:
Brucella melitensis*;*
Brucellosis

關鍵詞：
馬爾他布氏桿菌；
布氏桿菌病

Hong Kong Med J 2005;11:403-6

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was negative. Chest X-ray, abdominal ultrasound, and computed tomography of the brain were unremarkable. Blood cultures taken on admission became positive on day 4 and yielded tiny gram-negative coccobacilli. Repeated blood cultures on day 4 yielded similar results. The isolate was identified as *Moraxella phenylpyruvica* by API 20NE (BioMérieux, Marcy l'Etoile, France) with the profile 1240004. The isolate was forwarded to the Public Health Laboratory Services Branch of the Department of Health and was identified as *Brucella* species by 16S RNA sequencing. The isolate was confirmed to be *Brucella melitensis* by the Centers for Disease Control and Prevention. Transthoracic echocardiogram for suspected infective endocarditis showed a 0.4-cm pericardial effusion but no valvular disease. Serum tube agglutination test for *Brucella abortus* was 1:163 840 and for *B melitensis* was 1:2560, performed on the specimen taken on day 1.

The patient commenced oral amoxicillin-clavulanate but undulating fever persisted. On day 5, therapy was changed to intravenous cefuroxime plus gentamicin; azithromycin was also added. All antibiotics were stopped on day 7 for a repeated sepsis workup because of persistent fever. The fever subsided gradually and subsequent blood cultures revealed resolution of bacteraemia. Upon the diagnosis of human brucellosis from preliminary sequencing result, oral doxycycline and rifampicin were started. The patient was discharged on day 20 and completed 6 weeks of oral rifampicin and doxycycline. A repeated echocardiogram was normal. At 2-month follow-up, the patient was well with normal liver function.

The patient recalled having handling and consuming goat placenta in Shenzhen 10 months prior to the onset of symptoms. She had manipulated the placenta on her own in the kitchen while preparing dinner for the family. Occupational and recreational history was otherwise unremarkable. Family members who consumed the cooked placenta remained asymptomatic.

Brucellosis is a well-documented laboratory-acquired infection. Before the presumptive identification of the isolates, most laboratory procedures that involved the isolate had been performed inside a biological safety cabinet. Laboratory staff who handled the isolate were asymptomatic and serological tests were unremarkable after 3 months.

Discussion

The patient had human brucellosis caused by *B melitensis*. The major sources of this infection are contaminated food and occupational contact. Cases of person-to-person transmission and accidental infection with live animal vaccines have been reported.[3,4] *Brucella* species are classified as potential bioterrorist agents and may be transmitted by this route.[1]

Laboratory workers are at risk when handling infected specimens or culture via aerosol-generating procedures or accidental inoculation of mucous membrane.[5] Despite the use of automated blood culture instruments, supplementary procedures—slide preparation for gram staining, subculture of the blood broth to agar plates, examination of plates for bacterial growth, and performance of biochemical, serological, and antibiotic susceptibility test—may result in direct exposure of laboratory technicians and contamination of the laboratory environment. All manipulation with live *Brucella* cultures and antigens should be performed using biosafety level-3 facilities.[6]

In this case, contact with infected goat placenta in Shenzhen was the most likely source of infection. In Mainland China, animal and human brucellosis occurs mainly in Shaanxi, Hebei, Henen, Shandong, Inner Mongolia, Liaoning, Xinjiang, Tibet, and Jilin. In Guangdong province, the infection rate for sheep, goat, cows, and swine is 1% to 5%; sporadic human cases are reported.[7] Analysis of *Brucella* strains isolated from humans and animals in China revealed that 73.79% were *B melitensis*, 13.64% were *B abortus*, and 5.78% were *Brucella suis*. *Brucella melitensis* was also the predominant strain associated with outbreaks.[7] Brucellosis has been rare in Hong Kong. With the increasing traffic between Hong Kong and China, more cases can be expected.

Brucellae are intracellular pathogens, mostly localised inside the reticuloendothelial system.[1] Symptoms usually occur within 2 weeks (sometimes up to 3 months) of infection, and include fever, chills, night sweats, headache, body aches, anorexia, and malaise. Localised disease may occur in many organ systems including the skeleton, urinary tract, central nervous system, liver, heart, and lung.[1] Our patient presented with fever and recurrent headache but few other signs. A non-specific clinical presentation of brucellosis together with the rarity of cases in developed areas often results in delayed diagnosis. Eliciting a travel and occupational history from patients may aid diagnosis.

A definitive diagnosis of brucellosis depends on

the isolation of brucellae. Blood, bone marrow, or tissue cultures are most often positive during the acute phase.[8] Biopsies of the bone marrow, liver, and lymph nodes typically show non-caseating granulomas. The erythrocyte sedimentation rate is of little diagnostic value. Common haematological findings include leukopenia, anaemia, and thrombocytopenia.[8] Modern automated blood culture systems have greatly improved the detection of the fastidious and slow-growing *Brucella* species. Use of some commercial systems allows detection of over 95% of cultures within the routine 7-day blood culture protocol.[9] However, prolonged culture for 21 days for suspected brucellosis cases remains a prudent approach.[1]

Misidentification of *Brucella* species as *M phenylpyruvica* by commercial galley-identification systems is well known and occurred in this case.[8] Suspected isolates should be sent for further confirmation. A low index of suspicion of brucellosis, failure to appreciate the fast-growing nature of the organism, and uncritical acceptance of commercial identification kit results were pitfalls in laboratory diagnosis. Other diagnostic tests available include enzyme-linked immunosorbent assay, serum agglutination test (SAT), and polymerase chain reaction.[1,8] A four-fold rise in SAT titer between the acute- and convalescent-phase samples is indicative of brucellosis. For a single specimen, an SAT titer of 160 or higher is suggestive of brucellosis.[1] Polymerase chain reaction is also being investigated as a more effective means of diagnosis and typing.[1,8] The 16S rRNA gene in bacteria is highly conserved within a species. By using broad-range primers, a 16S rRNA gene sequence can be readily obtained, leading to rapid confirmatory identification of an unknown isolated *Brucella* species by simple comparison with the consensus sequence in database (eg GenBank). A critical advantage of sequencing is its rapidity that allows quick implementation of control and preventive measures in an emergency biothreat situation and timely treatment for affected patients. Simplification of steps in sequencing minimises the biosafety hazard involved in laboratory identification. Sequencing also allows yield of alternative identification without the need for any additional laboratory work. In contrast, polymerase chain reaction tests either positively identify gene regions associated with a *Brucella* species or exclude *Brucella* species.[10]

The optimum therapeutic approach to brucellosis remains a subject of debate despite years of research. Treatment depends on the patient's age and pregnancy status. The WHO expert panel on brucellosis recommends rifampicin 600 to 900 mg and doxycycline 200 mg daily for a minimum of 45 days for treatment of acute disease in adults. In children, rifampin has been recommended, with trimethoprim or sulfonamide as alternatives if the case is straightforward.[8] Combination therapy was important to reduce the relapse rate and treatment failure. Relapse is common if therapy is discontinued prematurely. Most relapses occur within 3 to 6 months of stopping therapy.[11] The patient in this report received gentamicin and azithromycin at some stage with a good clinical response. Nonetheless, a previous study reported a high rate of therapeutic failures and relapses in patients prescribed a regimen of 3 weeks' azithromycin and gentamicin.[12]

Control of human brucellosis depends on control of the disease in the animal reservoir, occupational precautions, and disinfection of dairy products and other potentially contaminated products. All available vaccines for human use have limited efficacy and some produce potentially serious side-effects. They play a very limited role in the prevention of human disease.[13]

Conclusion

Human brucellosis may be unsuspected because of the non-specific symptoms and its rarity in Hong Kong. Eliciting the travel and occupational history of a patient is essential to aid clinical diagnosis. Subsequent appropriate antimicrobial therapy can reduce morbidity, and prevent complications and relapses. Brucellosis should remain one of the differential diagnoses in the presence of prolonged and unexplained fever. It is a well-known laboratory-acquired infection, and manipulation of live *Brucella* cultures and antigens should be performed using biosafety level-3 facilities. This case highlighted the fact that early recognition of brucellosis facilitates the treatment process and protects laboratory staff from laboratory-acquired infection.

Acknowledgements

We thank Public Health Laboratory Services Branch of the Department of Health and the Centers for Disease Control and Prevention for confirmation of isolate identity.

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