CASE REPORT

FIRST CASE REPORT OF BRUCELLOSIS IN A CHILD IN THAILAND

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Abstract. Brucellosis is uncommon in children. In Thailand, there have been only seven adult cases reported, all with *Brucella melitensis*. We describe here the first reported pediatric case of brucellosis in Thailand. A 12-year old boy presented with prolonged fever for one month, pancytopenia, pneumonia and peritonitis. The blood culture grew out *Brucella melitensis*. He responded well to combination therapy consisting of doxycycline and gentamicin. He recovered fully without relapse during the 6 month follow-up.

Keywords: *Brucella melitensis*, brucellosis, children, Thailand

INTRODUCTION

Brucellosis is a zoonotic disease transmitted to humans by infected animals, mostly through direct animal contact or consumption of animal products (Eckman, 1975; Malik, 1997; Mantur *et al*, 2007). Transmission is also believed to occur via inhalation of airborne animal manure particles (Williams, 1970; Mantur *et al*, 2007). Four species of *Brucella* are known to cause human disease: *B. melitensis*, *B. abortus, B. suis*, and *B. canis* (Pickering *et al*, 2012). *B. melitensis* is the most common cause of brucellosis in humans and causes more severe disease than the other species (Mantur *et al*, 2004). Goats, sheep and camels are the main animal hosts of *B. melitensis*. In rare cases, human brucellosis has been caused by marine mammal *Brucella* (Corbel, 1997; Sohn *et al*, 2003; McDonald *et al*, 2006). The protean manifestations of brucellosis make it difficult to make a clinical diagnosis (Hatipoglu, 2004; Mantur *et al*, 2004, 2006; Shaalan *et al*, 2002). This report describes the first case of laboratory confirmed brucellosis in a child in Thailand.

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CASE REPORT

A 12-year-old previously healthy boy presented at Sam Roi Yot Hospital, Prachuap Khiri Khan Province, with fever for one month. At the onset, the fever was

low grade, usually at night, and accompanied by arthralgia, myalgia, and frequent epistaxis. Three days prior to admission at Siriraj Hospital, Mahidol University, Bangkok, he developed high fever and pallor which prompted his parents to seek medical attention.

He was admitted at Siriraj Hospital because of prolonged fever. On admission, he appeared ill and pale but was alert and oriented. His body temperature was at 38.5ºC, his respiratory rate was 20/min, his heart rate was 112/min, and his blood pressure was 92/64 mmHg. His weight and height were 30 kg (10^{th}-25^{th} percentile), and 149.3 cm (50^{th}-75^{th} percentile), respectively. He had a left cervical palpable lymph node 1 centimeter in diameter. He had non-tender hepatosplenomegaly with a liver palpable 4 centimeters below the right costal margin, and the spleen palpable 3 centimeters below the left costal margin. No other abnormalities were detected. He had been living on a pineapple farm in Sam Roi Yot District, Prachuap Khiri Khan Province, southern Thailand. Additional history revealed there were many goat farms in the area, and he had contact with goats approximately 2 months prior to the onset of fever through holding goats for immunization at his uncle's farm.

The complete blood count (CBC) revealed a hemoglobin of 8.5 g/dl, a hematocrit of 26.8%, a MCV of 73.8 fl, a MCH of 23.4 pg, a MCHC of 31.7 g/dl, a RDW of 16.8%, a white blood cell count (WBC) of 4,230 cells/mm^3 with 38% neutrophils, 58% lymphocytes, and 3% monocytes and a platelet count of 90,000/mm^3. His urinalysis was normal. Blood chemistry revealed normal electrolytes, renal function and liver function with an alanine transaminase of 73 U/l and aspartate transminase of 31 U/l. A chest x-ray showed bilateral interstitial infiltrates. Additional laboratory investigations on admission included serology for Epstein-Barr virus, human immunodeficiency virus, cytomegalovirus, *Burkholderia pseudomallei*, and *Orientia tsutsugamushi*, as well as blood smears for malaria, a tuberculin skin test, and a sputum acid-fast stain. The results were later found to be negative. To investigate the cause of the anemia and thrombocytopenia, a bone marrow biopsy was performed, which revealed normal cellularity and maturation, infrequent hemophagocytosis, no clusters of blasts or abnormal lymphoid cells, a slight increase in the number of megakaryocytes, a decrease in iron deposition, and no ring sideroblasts. The bone marrow examination was also negative for acid-fast bacilli, fungi, malignancy or granulomas. A blood culture was obtained on admission and he was put on cefotaxime 100 mg/kg/day as empirical treatment.

He continued to have high grade fever to a maximum of 39.8ºC. On day 5 of hospitalization he developed abdominal pain with guarding and rigidity suggesting peritonitis. A computerized tomogram revealed bilateral pleural effusions, ascites, hepatosplenomegaly and mesenteric lymphadenopathy. Abdominal paracentesis revealed 50 ml of clear yellow fluid. Peritoneal fluid examination revealed a red blood cell count of 1,600/mm^3, a WBC count of 110/mm^3 with100% lymphocytes, a glucose level of 101 mg/dl, a LDH of 724 U/l and an albumin of 2.1 g/dl. A Gram stain and acid-fast stain of the peritoneal fluid were both negative for organisms, and a culture of the fluid failed to detect bacteria, fungi or mycobacteria. A polymerase chain reaction (PCR) of the peritoneal fluid for tuberculosis was negative. A repeat CBC revealed a hemoglobin of 9 g/dl, a hematocrit at 29.3%, a WBC

count of 4,100/mm^3 with 43% neutrophils, 57% lymphocytes and 1% monocytes, and a platelet count of 87,000/mm^3. A prothrombin time and partial thromboplastin time were normal. A fibrinogen level was 231.3 mg/dl (normal 200-400) and the D-dimer was 5,379.8 μg/l (normal < 500 μg/l). The antibiotic regimen was empirically changed to piperacillin/tazobactam 300 mg/kg/day to improve coverage of an intra-abdominal infection.

On day 8 of hospitalization, the patient remained febrile and had no signs of improvement. Blood cultures taken on admission examined with the automate system (BacT/Alert) revealed growth of small gram-negative aerobic bacilli, suspected to be *Brucella* sp. This finding prompted a change in therapy to oral doxycycline 100 mg twice daily and intravenous gentamicin 5 mg/kg/day. The patient responded well to the treatment. The signs of abdominal peritonitis disappeared within 48 hours and the fever subsided by 72 hours of treatment. Blood cultures taken on days 4 and 7 of hospitalization also grew the same organism, which was later identified as *Brucella melitensis* using biochemical tests and the automated Vitek 2 system. The organism was also confirmed to be *Brucella* sp by 16S rDNA sequencing. The minimal inhibitory concentrations (MIC) for trimethoprim-sulfamethoxazole and gentamicin determined by the E-test were 0.032 μg/ml and 0.19 μg/ml, respectively. He recieved 2 weeks of daily gentamicin and 6 weeks of doxycycline. By 4 weeks of treatment, he had complete clinical recovery and a normal CBC. He continued to do well for 6 months follow-up after therapy was completed.

DISCUSSION

Brucellosis is a multisystemic disease with many clinical presentations (Hatipoglu *et al,* 2004). Brucellosis in children is frequently mild, self-limited, and less likely to be chronic compared to adults (Shaalan *et al,* 2002). Brucellosis in adults usually causes fever (75.5%), night sweats (69.7%) and arthralgia (75.5%) (Hatipoglu *et al,* 2004). The most common symptoms in children are fever, malaise/myalgia and arthralgia (al-Eissa and al-Nasser, 1993; Mantur *et al,* 2004; Giannakopoulos *et al,* 2006). The most common sign in children is hepatomegaly, followed by splenomegaly and lymphadenopathy (Giannakopoulos *et al,* 2006); all were found in our case. The most common complications involve bone and joint, particularly peripheral arthritis in adult (Mousa *et al,* 1987) and spondylitis in the older age (Colmenero *et al,* 1996). The osteoarticular involvement in children was monoarticular predominantly affecting hips or knees (al-Eissa *et al*, 1990; Benjamin *et al*, 1992; Bosilkovski *et al,* 2013). The infection may involve several organ systems, including hematologic, genitourinary, gastrointestinal, hepatobiliary, cardiovascular, nervous, and respiratory systems (Mantur *et al,* 2001; Kantarçeken *et al,* 2005; Mantur *et al,* 2006; Ozisik *et al,* 2006). There have been reports of unusual presentations, such as neurobrucellosis, pericarditis, pancytopenia, epididymoorchitis, uveitis, mixed cryoglobulinemia with renal failure, cutaneous vasculitis and peritonitis (Hatipoglu *et al,* 2004; Hermida Lazcano *et al,* 2005; Dizbay *et al,* 2007). Fatal outcomes occur due to extensive vasculitis (Dizbay *et al,* 2007) often accompanied by encephalopathy (Caksen *et al,* 2003).

Hematologic alterations in brucellosis are common (Martin-Moreno *et al,* 1983; Crosby *et al,* 1984; Aysha and Shayib, 1986). A large prospective study in adults found

leukopenia and relative lymphocytosis in 28.7% (152/530) of cases (Colmenero *et al*, 1996). Another common hematologic finding is mild anemia (al-Eissa and al-Nasser, 1993). Pancytopenia has been found in 5-20% of pediatric cases (al-Eissa and al-Nasser, 1993; al-Eissa *et al*, 1993; Yildirmak *et al*, 2003; Karakukcu *et al*, 2004). The causes of the pancytopenia may be multifactorial, including hemophagocytosis, hypersplenism, bone marrow hypoplasia, bone marrow granulomas, and immune destruction (Schirger *et al*, 1960; Crosby *et al*, 1984; al-Eissa and al-Nasser, 1993; Colmenero *et al*, 1996; Yildirmak *et al*, 2003; Karakukcu *et al*, 2004). Our case presented with initial anemia and thrombocytopenia, and only later in the course he developed pancytopenia. Hematological changes in brucellosis typically resolve promptly with treatment (Colmenero *et al*, 1996) as was the case with our patient. Spontaneous peritonitis in brucellosis is quite rare and has been reported mainly among adult patients with chronic liver disease (Demirkan *et al*, 1993; Halim *et al*, 1993; Alcalá *et al*, 1999; Erbay *et al*, 2003; Gençer and Ozer, 2003; Gürsoy *et al*, 2003; Hatipoglu *et al*, 2004; Kantarçeken *et al*, 2005; Ozisik *et al*, 2006). We are unaware of any reports of spontaneous peritonitis among children with brucellosis and believe our patient is the first reported case. Pulmonary manifestations in brucellosis occur in about 16% in adults (Pappas *et al*, 2003) and include bronchopneumonia, cavitating pneumonia, pulmonary nodules, hilar lymphadenopathy, empyema and pleural effusions (Colmenero *et al*, 1996). Our patient had bilateral infiltrations and pleural effusions, but without recognizable respiratory symptoms.

The gold standard for diagnosing brucellosis is blood cultures. Our patient had four blood cultures performed on days 0, 4, 7 and 14 of hospitalization, all grew out gram-negative coccobacilli after 3 days incubation in an automated system. A blood culture obtained one month later finishing treatment was negative. As a facultative intracellular pathogen, cultures of bone marrow for *Brucella* typically have a higher yield than blood cultures (Gotuzzo *et al*, 1986; Ozkurt *et al*, 2002; Tsolia *et al*, 2002; Hatipoglu *et al*, 2004; Karakukcu *et al*, 2004; Mantur *et al*, 2006). We did not perform a bone marrow culture in our patient since we did not suspect brucellosis in this child at the time it was performed.

Treatment of acute brucellosis requires combination regimens that result in fewer failures than monotherapy (Skalsky *et al*, 2008). The World Health Organization (Anonymous, 1986; Corbel, 2006) recommends doxycycline and rifampicin daily for a minimum of 6 weeks. Alternatively, rifampicin can be replaced with streptomycin, administered intramuscularly for only 2 weeks (Anonymous, 1986; Corbel, 2006). However, a recent review of 30 randomized controlled trials (Skalsky *et al*, 2008) found that the doxycycline with rifampicin regimen has significantly higher relapse rates than doxycycline with streptomycin. However, doxycycline with streptomycin is not as effective as doxycycline with rifampin and an aminoglycoside (triple drug regimen) (Skalsky *et al*, 2008). In that review (Skalsky *et al*, 2008), gentamicin was not inferior to streptomycin and could be given intravenously. A quinolone with rifampin was found to be not as effective as doxycycline in combination with either rifampicin or streptomycin. In our patient, the susceptibility test revealed *B. melitensis* was sensitive to both gentamicin and trimethoprim-sulfamethoxazole. However, trimethoprim-sulfamethoxazole

has been reported to have higher relapse rates and is usually used in triple drug regimens (Mantur *et al*, 2007). We thus opted to treat our patient with gentamicin for 2 weeks and doxycycline for 6 weeks. He responded rapidly and had no signs of relapse during 6 months of follow-up.

Goats remain the main source of *B. melitensis* (Corbel, 1997). A review of 7 adult cases of brucellosis caused by *B. melitensis* in Thailand during 1970 to 2005 revealed 29% (2/7) had a history of consuming non-pasteurized goat milk and 57% (4/7) had a history of contact with goats (Paitoonpong *et al*, 2006). In our case, we hypothesize that the mode of contraction was direct contact with infected goats.

In conclusion, we report here the first case of brucellosis in a child in Thailand whose case included peritonitis. Brucellosis has a broad range of signs and symptoms and is difficult to diagnose based on clinical findings alone. In non-endemic areas, such as Thailand, brucellosis is a diagnostic challenge. Failure to recognize brucellosis and provide appropriate antibiotic treatment may result in serious complications or death. It is important to include brucellosis in the differential diagnosis of children with prolonged fever and hepatosplenomegaly, especially in those with a history of exposure to animals.

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