

Infective endocarditis caused by *Brucella melitensis*: a case report highlighting the importance of history taking and laboratory analysis

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Abstract. We present a case of *Brucella* endocarditis in a 13 year old patient with known aortic stenosis. She was admitted to the National Heart Institute/Institut Jantung Negara, Malaysia with complains of fever, pain and swelling of left knee. Transthoracic echocardiography and transesophageal echocardiography showed no evidence of vegetations on the aortic valve. Differential diagnosis was made based on clinical manifestations, positive serology tests and isolation of *Brucella melitensis* from blood culture. The patient has a history of consumption of unpasteurised goat's milk prior to clinical symptoms. Although rare, the case emphasize that *Brucella* could be a potential complication of infective endocarditis (IE) involving patient who consumed unpasteurised goat's milk. The diagnosis of bacterial endocarditis based on clinical findings and supported by laboratory results has led to the appropriate treatment of this patient. To the best of our knowledge, this is the first case reported for *Brucella* endocarditis in Malaysia.

INTRODUCTION

Brucellosis is transmitted to humans through consumption of unpasteurised dairy products, inhalation of infected aerosolized particles and through direct contact with infected animal parts (Corbel, 2006). Common clinical findings of the disease are fever, arthralgia and myalgia which may progress to affect multiple organs and system of the human body (Buzgan *et al.*, 2010). Epidemiological findings from patients are of importance to facilitate an early diagnosis of suspected *Brucella* cases (Corbel, 2006).

Brucella endocarditis is a rare manifestation in clinical cases and occurs in less than 2% of patients (Buzgan *et al.*, 2010; Çalik & Gökengin, 2011). However, it has been reported to occur in up to 95% of

cases in the Mediterranean regions known to be endemic with brucellosis (Keshtkar-Jahromi *et al.*, 2012). Cardiovascular manifestation in the form of infective endocarditis (IE) is the most common cause of death from brucellosis (Corbel, 2006; Koruk *et al.*, 2012).

The present report described a case of aortic endocarditis due to *B. melitensis* in a young adolescent in Malaysia. The case study was presented with the aim to share information on the diagnosis of bacterial IE which was supported by clinical manifestations and laboratory results.

CASE REPORT

A 13-year old girl presented to Institut Jantung Negara (IJN) with intermittent fever

of one month duration and associated with swelling and pain of the left knee. There was also history of loss of weight and appetite. She did not have any symptoms suggestive of heart failure. Prior to admission, she was treated with oral cephalexin by a private practitioner but her symptoms persisted. She had congenital aortic stenosis and had undergone balloon valvuloplasty at 2 years of age. Prior to this episode of fever, she has been well. On admission to the IJN, the patient was found to be febrile, 38°C, blood pressure 103/53 mmHg and pulse rate was 71 beats/min. There was collapsing pulse and an early diastolic murmur suggestive of aortic regurgitation. The left knee was swollen, tender on movement and had decreased mobility.

On admission, the total white blood cell count was $7.5 \times 10^9/L$, hemoglobin was 9.9 g/dL full blood picture confirmed the presence of hypochromic, microcytic anaemia and platelet count was $409 \times 10^9/L$. Erythrocyte sedimentation rate (ESR) was raised 23 mm/h but the C-reactive protein was 2.7 mg/L and the urine examination was normal. The work up for rheumatic fever and rheumatoid arthritis were negative. She did not have any other signs to suggest IE (Osler nodes, Roth spots, Janeway lesions). She was not tachypnoeic, not in heart failure and was mentally alert. The working diagnosis was probable IE and left septic arthritis in the presence of aortic regurgitation post aortic balloon valvuloplasty. Echocardiogram confirmed the presence of severe aortic regurgitation but no vegetation was seen on transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE).

Blood culture was performed three times and all the Gram-stain of the blood cultures showed Gram-negative coccobacilli after 3 days of aerobic incubation in BACTEC bottle at 37°C. The culture on the blood agar plate did not yield any positive results at 72 h. However after 10 days post incubation, small greyish colonies with no lysis were isolated. Standard microbiological tests of the isolate showed results of Gram-negative coccobacilli that were catalase, oxidase and urease positive. Production of hydrogen

sulfide was not observed. These were key tests for *Brucella* sp. identification (Garrity *et al.*, 2005). Identification using Vitek 2 system (bioMérieux, Durham, USA) did not yield any identity.

The isolate was also subjected to laboratory diagnosis by molecular methods. Polymerase chain reaction (PCR) was performed on the isolate and a 223 base pair PCR product was observed using the primers amplifying a gene encoding a 31 kDa *Brucella abortus* antigen (BCSP31) (Baily *et al.*, 1992; Kamal *et al.*, 2013). PCR amplification of 16S rRNA of the isolates was also carried out using universal 16S rDNA primers U1F (5'-CTYAAAKRAATTGR CGGRRRSC-3' and U1R (5'-CGGGCGG TGTGTRCAARRSSC-3') (Rivas *et al.*, 2004) and the PCR product was sent for sequencing analysis. Resulting sequences were compared with known *Brucella* sequences deposited in GenBank by using the Basic Local Alignment Search Tool (BLAST) program (National Center for Biotechnology Information, Bethesda, Maryland). The organism was identified as *B. melitensis* with a match of 98%. Determination of the species of *Brucella* was also carried out using high resolution melting (HRM) analysis as described by Mohamed Zahidi *et al.* (2015). The HRM analysis showed that the *Brucella* isolate was *Brucella melitensis*.

The presence of *Brucella* antibody was detected using commercial *Brucella* immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assay (ELISA) kits (Vircell SL, Granada, Spain). A positive result with antibody index of 28.6 and 24.0 (cut-off index of 11 and above) was noted for IgM and IgG ELISA respectively. Brucella-capt test (an immunocapture-agglutination technique) (Vircell SL, Granada, Spain) was performed as specified by the manufacturer and the result was also positive with the titre of 1:5120 (cut-off value of 320).

As our initial working diagnosis was probable IE with left septic arthritis in the presence of aortic regurgitation post aortic balloon valvuloplasty, the patient was empirically started on intravenous high dose penicillin 1.8 megaunits 6 hourly and

gentamicin 40 mg 8 hourly while awaiting blood culture results. The patient was on these antibiotics for three weeks until the confirmatory diagnosis of *Brucella* endocarditis was made based on the PCR and serology results.

Once diagnosis of brucellosis was confirmed, her antibiotics were changed to doxycycline 100 mg 12 hourly and rifampicin 600 mg daily for four months. Intravenous gentamicin was continued for a total of four weeks duration. The duration of antibiotics were given as advised by the Infectious Diseases Consultant, to prevent long term sequelae and relapses. Following completion of antibiotics, she has remained afebrile, her joint swelling and pain had resolved and she has resumed her normal activities.

During the patient's follow-up one year later, her C-reactive protein and ESR normalized. The ESR decreased from 23 mm/h to 9 mm/h and the total white blood cell count was $8.8 \times 10^9/L$ (neutrophils, 33.5%; lymphocytes 57%). The knee swelling and inflammation had resolved without any intervention. Her TTE findings demonstrated severe aortic regurgitation and there were no vegetations seen. The serological testing of her serum remained positive with antibody index of 18.9 and 16.9 (cut-off index of 11 and above) for IgM and IgG ELISA

respectively. Brucellacapt test showed a negative titre of 1:160 (cut-off value of 320). She subsequently underwent successful aortic valve repair in February 2016. The repeat serological testing 14 months after completion of antibiotics still remained positive with antibody index 19.9 and 20.7 for IgM and IgG respectively. However, the Brucellacapt test remained negative and patient did not have clinical symptoms or signs suggestive of a relapse. The serologic test results and antibiotic duration of the patient are shown in Table 1. Figure 1 shows the timeline of the patient's symptoms, therapies, investigations, laboratory analysis and outcomes.

DISCUSSION

Brucella endocarditis cases commonly affect the aortic valve and usual echocardiography findings include valve vegetations (Çalik & Gökengin, 2011; Koruk *et al.*, 2012). The diagnosis of possible IE by *Brucella* sp. in the present case was based on proposed modifications of Duke criteria for diagnosing IE (Li *et al.*, 2000; Habib *et al.*, 2015). One major criterion met was two positive blood cultures obtained from blood samples drawn > 12 h apart and one minor criterion met was the predisposing heart

Table 1. Serologic test results and antibiotic duration of patient from October 2014 to May 2016

Period	Serology results		Antibiotic duration
	ELISA ^a	Brucellacapt ^b	
On admission	IgM: 28.6; IgG: 24.0	1:5120	Penicillin and gentamicin for 3 weeks
Probable <i>Brucella</i> endocarditis	–	–	Gentamicin continued for a total of 4 weeks Doxycycline and rifampicin for 4 months
Post completion of antibiotics	IgM: 18.9; IgG: 16.9	1:160	–
Fourteen months post completion of antibiotics	IgM: 19.9; IgG: 20.7	1:160	–

ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin.

^aELISA: Cut off-titre for antibody index ≥ 11 , positive; ^bBrucellacapt: Titre $\geq 1:320$, positive.

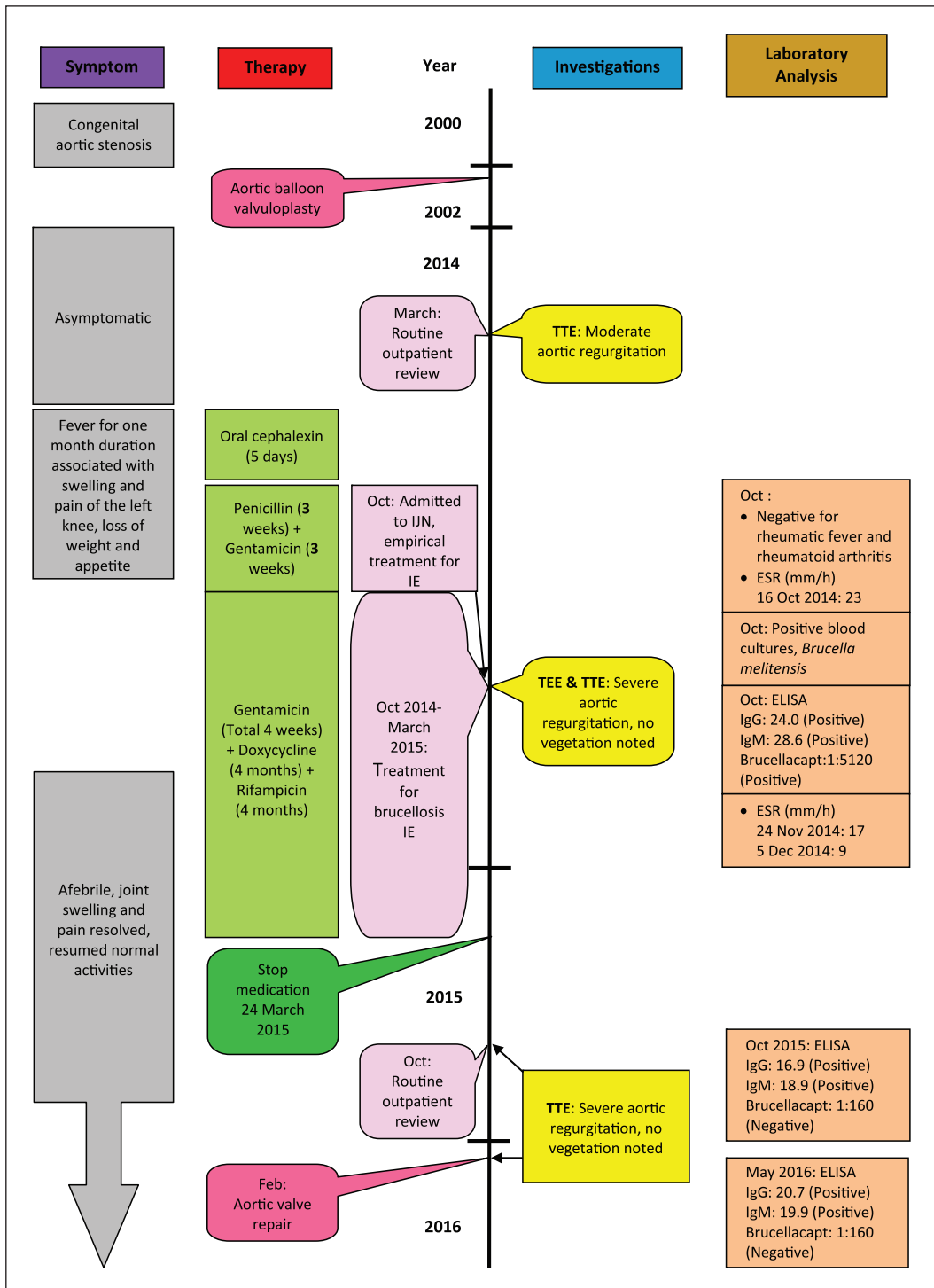


Figure 1. Timeline of symptoms, therapies, investigations, laboratory analysis and outcomes. Abbreviations: ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate; IE, infective endocarditis; Ig, immunoglobulin; IJN: Institut Jantung Negara; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography.

condition. The clinical history and clinical findings from the patient were also suggestive of possible IE even though there were no vegetations in the heart.

In a retrospective study conducted on 308 cases of *Brucella* endocarditis, more than 80% of patients were diagnosed with *B. melitensis* and 14% were infected by *B. abortus*, where most of the cases were from the Middle East and the Mediterranean regions (Keshtkar-Jahromi *et al.*, 2012). Malaysia which is non-endemic to brucellosis reported a total of 79 brucellosis cases due to an outbreak in year 2011. Clinical manifestations for most cases were unexplained chronic fever and none of the cases presented with *Brucella* endocarditis (Leong *et al.*, 2015). However, in year 2014 we received a culture sample from a patient admitted to IJN which led to our first reported case of probable IE due to *B. melitensis*.

The symptoms and signs of brucellosis are non-specific, therefore a combination of various laboratory test results is crucial for definite diagnosis. For example, the usage of automated blood culture systems has reduced the time needed for isolation and identification of *Brucella* sp. (Raj *et al.*, 2014). In the present report, we are unable to identify the isolate using Vitek 2 system but we managed to identify the bacteria using molecular methods such as PCR. PCR is not only highly sensitive and specific but also allow differentiation of species and strains within the genus *Brucella* (García-Yoldi *et al.*, 2006; López-Goñi, *et al.*, 2011). Furthermore, molecular techniques reduced many subculturing procedures which may expose the laboratory staff to this biosafety level 3 bacteria.

Serological testing such as serum agglutination test and immunoenzymatic assays for detection of antibody against *Brucella* are important laboratory diagnostic tests for brucellosis (Park *et al.*, 2012). In this case, the serological results showed high antibody index for both IgM and IgG. The positive serological results is further complemented by Brucellacapt testing which has been reported useful in the follow up test for patients with brucellosis (Mile

et al., 2010; Özdemir *et al.*, 2011). *Brucella* antibodies still remained high one year after completion of antibiotics despite no clinical symptoms and signs of infection and a negative Brucellacapt test. It has been shown that the *Brucella* antibodies can remain persistently high for up to a median 18.5 months after successful treatment in patients with a previous history of acute brucellosis who are clinically cured (Corbel, 2006; Memish *et al.*, 2002). Therefore in these patients it is advisable to do additionally serological tests example Brucellacapt and/or repeat the PCR for *Brucella* culture to determine if the patient has any relapse. This prevents unnecessary overdiagnosis and antibiotic treatment. In this study the antibody test by Brucellacapt was negative on follow-up, showing that the patient had responded to treatment.

The patient was initially treated as suspected bacterial endocarditis even though she did not have any vegetations on the heart valves. This was based on the history of prolonged fever and acute worsening of the aortic regurgitation on transthoracic echocardiogram. Positive cultures were only obtained from her blood agar plate on day 10 post incubation, highlighting the importance of prolonging the incubation time and pursuing further testings (serology and PCR) in patients with high clinical suspicion of *Brucella* sp. infection. Despite resolution of the joint pain and fever with the penicillin and gentamicin, we changed the antibiotics to a combination of doxycycline and rifampicin for a duration of four months once the diagnosis of brucellosis was confirmed. It has been recommended that a combination of doxycycline with either rifampicin or streptomycin for a duration of 8–12 weeks in addition to gentamycin for 2–4 weeks would decrease the risk of inadequate treatment, complications and relapses (Alici *et al.*, 2014; Corbel, 2006; Habib *et al.*, 2015).

In conclusion, we emphasized that when there is no evidence of endocardial involvement on echocardiogram, high suspicion of *Brucella* endocarditis should be supported by relevant history, clinical symptoms and a combination of serology

testing and positive blood culture. The laboratory results, namely high titres of serum and also the isolation of *B. melitensis* from the blood culture are strong clinical indicators which aided in the diagnosis of *Brucella* endocarditis in our case. Close follow-up and regular monitoring for complications and relapses are also essential prerequisites for medical management of patients with *Brucella* endocarditis.

ABBREVIATIONS

ELISA: enzyme-linked immunosorbent assay; ESR: erythrocyte sedimentation rate; HRM: high resolution melting; Ig: immunoglobulin; IJN: Institut Jantung Negara; IE: infective endocarditis; PCR: polymerase chain reaction; rDNA: ribosomal deoxyribonucleic acid; TEE: transesophageal echocardiography; TTE: transthoracic echocardiography.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor of this journal.

CONTRIBUTIONS

BYT designed the case report and drafting the manuscript. GK was responsible for the patient's management, collected all significant clinical information and coordinated drafting the manuscript. BYT, RH, JMZ, SSMS, HH and NAD participated in the bacterial identification and serological testing of the samples. NA reviewed, edited and supervised the manuscript. All authors have read and approved the final version.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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