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## Clinical human brucellosis in Malaysia: a case report

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## PEER REVIEW

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## Comments

This is an interesting work, where the authors report on human infection by *B. melitensis* which is helpful for clinical diagnosis and treatment.

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## ABSTRACT

Clinical human brucellosis is quite rare in Malaysia although seroconverters are relatively more. This report describes a case of clinical human brucellosis in Malaysia. This case involved a 29-year-old research assistant in a veterinary microbiology laboratory. She complained of intermittent fever, anorexia, profuse sweating, malaise, headache, normotensive (110/60 mm Hg), muscle pain, and arthralgia for 3 d. Blood tests against dengue and malaria were negative thus she was prescribed vitamin C, paracetamol and cough syrup for common flu. The complaints, however, persisted on and off for the next 1 month. She eventually developed anemia and hypotension (90/50 mm Hg) and started to show reduced body weight. Abdominal palpations revealed hepatomegaly and splenomegaly with pain. Thus, brucellosis was suspected before the Rose–Bengal plate test was performed, which revealed the presence of high level of antibody against *Brucella*. The same test was repeated after 14 d and the results confirmed the presence of high antibody level against *Brucella*. Following serum agglutination test, a diagnosis of brucellosis was made and she was eventually prescribed rifampicine *p.o.* once a day combined with doxycycline *p.o.* twice a day for 6 consecutive weeks before she made a full recovery.

## KEYWORDS

Brucellosis, Human, Malaysia

## 1. Introduction

Brucellosis is a multisystem disease with a broad spectrum of non-specific symptoms that generally occur within 2 weeks but sometimes up to 3 months after inoculation. In human, it developed as a result of consuming unpasteurized, contaminated goat's milk or soft cheese that had been infected with *Brucella melitensis* (*B. melitensis*) [1]. This Gram-negative, aerobic non spore forming coccobacillus is a free-living, soil-dwelling organism that usually infects goats and sheep. In infected hosts, the bacterium appears as intracellular localization,

particularly within the reticuloendothelial system [2]. There are six species of *Brucella* and four of which are known to infect humans. They are *B. melitensis*, *Brucella abortus* (*B. abortus*), *Brucella suis* (*B. suis*) and *Brucella canis* (*B. canis*). *B. abortus* is found principally in cattle, *B. melitensis* in goats and sheep, *B. suis* in swine and *B. canis* in kennel-raised dogs [3].

Both human and animal brucellosis are still endemic in some parts of the world such as Pakistan [4], India [5], China [6] and Sri Lanka [7]. About 500 000 new brucellosis cases were reported annually from all over the world [8]. In Malaysia, *Brucella* among animals was first isolated in

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1950 and the government embarked upon an eradication program for brucellosis (National Surveillance Program for Animal Brucellosis) in cattle, buffaloes, goats and sheep since 1978<sup>[9]</sup>. Brucellosis in human was first reported in 2010 involving a seven-year boy after consumed a raw milk of an infected goat. Nevertheless, studies have shown seropositive humans in Malaysia, mainly among veterinarians and farmers associated with occupational exposure to animals. The seroprevalent was mainly males (90%) within the age range between 20 and 45 years old<sup>[10]</sup>. This paper reports a case of human brucellosis involving a laboratory assistant in Malaysia.

## 2. Case report

A 29-year-old woman who worked as a research assistant in a veterinary microbiology laboratory complained of intermittent fever, anorexia, profuse sweating, malaise,

headache, normotensive (110/60 mm Hg) and muscle pain especially of the neck and shoulder, and arthralgia for 3 d. She was one of the 9 laboratory workers who were involved in a research on isolation of *B. melitensis* from goats for the past 3 months. The next day, she was admitted to a clinic for blood tests against dengue and malaria but was found negative. Therefore, a common flu was suspected and she was prescribed vitamin C, paracetamol and cough syrup.

The complaints, however, persisted on and off for the next one month and the conditions were getting worse. Anemia and hypotension (90/50 mm Hg) were eventually developed and she started to show reduced body weight from 50 kg to 43 kg. Abdominal palpations revealed hepatomegaly and splenomegaly with pain. The hemoglobin, hematocrit and neutrophil were low while the lymphocytes and erythrocyte sedimentation rate were high during the first month of infection. Following treatment (Tables 1 and 2), the conditions improved slightly.

**Table 1**

The haemogram results of the patient during *Brucella* infection.

Date	RBC CMM×10 <sup>3</sup>	Hb g/L	PCV L/L	MCV fL	MCHC %	WBC CMM×10 <sup>3</sup>	B. NEUT %	S. NEUT %	LYMP %	MONO %	EOS %	BAS ×10 <sup>12</sup> /L	THROM ×10 <sup>12</sup> /L	PP g/L	II Unit	ESR MM/HR
21-01-2013	–	12.7	40.2	–	–	5.51	–	58	32	8.0	1.0	1	184	–	–	–
29-01-2013	3.93	10.9	33.6	85.4	32.6	4.88	–	51	37	10.0	1.0	1	222	–	–	64
01-02-2013	4.04	11.4	35.0	87.0	32.6	4.63	0.14	22	18	3.7	1.0	0	260	90	2	–
04-02-2013	–	11.2	34.4	–	–	3.80	–	–	–	–	–	–	310	–	–	–
03-04-2013	3.98	11.2	34.0	85.0	32.9	4.18	0.04	23	14	2.1	1.0	0	189	74	2	–
16-04-2013	4.42	12.5	37.8	85.5	33.0	4.79	–	50	39	7.0	3.0	1	252	–	–	34
02-01-2013	4.41	12.3	38.1	–	–	4.62	–	42	47	–	–	–	232	–	–	–

RBC: Red blood cell count, Hb: Haemoglobin B, PCV: Packed-cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell count, NEUT: Neutrophil, LYMP: Absolute lymphocyte count, MONO: Monocytes, EOS: Eosinophil, BAS: Basophils, THROM: Thrombocytes, ESR: Erythrocyte sedimentation rate.

**Table 2**

The blood biochemistry results of the patient during *Brucella* infection.

Date	TP g/L	AST U/L	ALT U/L	Urea mmol/L	Creatinine umol/L	Tbil umol/L	Albumin g/L	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L
29-01-2013	62.9	33.0	26.0	2.43	76	8.0	38.2	133.0	3.5	102.0
03-04-2013	66.5	17.9	9.9	4.30	79	3.8	42.4	138.6	5.0	103.4
16-04-2013	–	21.0	14.0	3.53	65	–	–	–	–	–

TP: Total protein, AST: Aspartate transaminase, ALT: Alanine aminotransferase, Tbil: Total bilirubin.

At this moment, either brucellosis or leptospirosis was suspected based on the symptoms and history of her previous handlings of both organisms. Therefore, the Rose-Bengal plate test (RBPT)<sup>[11]</sup> was performed using antigen of *B. melitensis*, which revealed the presence of high level of antibody. The same test was repeated after 14 d and the results confirmed the presence of high antibody level against *B. melitensis* (Table 3). Eventually, standard agglutination test was performed<sup>[12]</sup>, which revealed the presence of high level of antibody titer against *Brucella* (1:40). The same tests were repeated after 14 d and the results confirmed the presence of high antibody level

against *Brucella* (Table 3).

Based on the serological tests and PCR, a diagnosis of brucellosis was made. She was eventually prescribed rifampicine *p.o.* once a day combined with doxycycline *p.o.* twice a day for 6 consecutive weeks.

**Table 3**

Results of Rose Bengal plate test (RBPT) prior to, during and post-treatment periods.

Date	Results	Description
31 January 2013 (Pre-treatment)	+++	Agglutination<3 seconds
4 April 2013 (During treatment)	++	Agglutination<15 seconds
16 June 2013 (Post-treatment)	+	Agglutination<30 seconds

### 3. Discussion

Brucellosis, particularly those caused by *B. melitensis* is among the most frequently reported laboratory-acquired infections. About 30% to 100% of cases of brucellosis in humans were obtained from laboratory exposures<sup>[13]</sup>. In this case, the patient was a microbiology laboratory worker, thus highly at risk of getting brucellosis through unpredicted exposure to cultures from clinical specimens. Transmission was believed to occur mainly via inhalation<sup>[14]</sup>. Nevertheless, human brucellosis appears to be quite rare in Malaysia, making the suspicion level among clinicians to be quite low.

RBPT has been proven as a cheap, rapid and effective serologic test compared to other serological tests, such as serum agglutination test, coomb test, compliment fixation test, indirect immunofluorescent antibody test and enzyme-linked immunosorbent assay (ELISA). With the minimum of equipment, RBPT can be performed and the results can be read by the naked eye. However, the specificity and sensitivity are unclear but the results are reliable<sup>[15]</sup>. In our study, serum agglutination test was also performed as second serology test to support the diagnosis. serum agglutination test had been recognised as the most commonly used test that is able to detect antibodies against *B. abortus*, *B. suis* and *B. melitensis* but not *B. canis*<sup>[12,16]</sup>.

This patient showed many of the acute symptoms of human brucellosis, which included undulating fever, myalgia and other clinical manifestations such as splenomegaly, hepatomegaly and spondylitis<sup>[17]</sup>. Infective endocarditis, although rare, is the most devastating complication from systemic brucellosis and could require surgical intervention. Splenic, hepatic and pulmonary abscesses can also develop. Lymphadenopathy is found in 10% to 20% of cases and splenomegaly or hepatomegaly in 20% to 30% of cases<sup>[18]</sup>. Other rare conditions include deep vein thrombosis, eukocytoclastic vasculitis, meningitis and nephritis. Ocular manifestations include optic neuritis, papilledema and uveitis<sup>[19]</sup>. Common hematologic findings include leukopenia, anemia, and thrombocytopenia<sup>[20]</sup>.

The standard treatment for human brucellosis is doxycycline 100 mg *p.o.* twice a day and rifampicin 450 mg/day *p.o.* for 6 weeks<sup>[21]</sup>. However, the standard treatments vary depending on the patient's age and pregnancy status<sup>[22]</sup>. There was no statistical difference regarding the type of combination therapy on the early clinical response of human brucellosis. Nevertheless, the recommended treatments are as follows. For children younger than 8 years, a combination therapy of trimethoprim-sulfamethoxazole and aminoglycoside<sup>[23]</sup> or a combination of rifampicin and trimethoprim-sulfamethoxazole for 45 d is needed<sup>[24]</sup>. For those aged 8 years and older, a combination of doxycycline and rifampin or a rifampicin and gentamicin combination could be prescribed<sup>[25]</sup>. Rifampicin combined with

ciprofloxacin for 30 d has also been shown to be effective and offers the advantage of a shorter-treatment duration<sup>[26]</sup>.

Guideline from the Center of Disease Control and Prevention (CDC) on management of laboratory exposures to *Brucella* spp. advises serological follow-up and antibiotic prophylaxis. The rationale for serological follow-up is to identify and treat asymptomatic infected patients, although the benefit is not proven. In one exposure incident, treating seroconverted asymptomatic workers led to milder symptoms compared with those treated for clinically apparent disease<sup>[18]</sup>. Prolonged follow-up of exposed workers is required, since the incubation period varies from 1 week to 5 months. Similarly, the benefit of early prophylaxis to the highest risk group was not confirmed since no seroconversion was produced in the high-risk staffs tested<sup>[27]</sup>.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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### Comments

#### Background

*Brucella* infections are rare in humans, despite being present in many animals used for consumption. The infection cases reported in humans is essential so that we can discuss investment in the management of infected animals, research laboratories safety and public health.

#### Research frontiers

This paper is a case report of human infection by *B. melitensis*, which is already known to be capable of infecting humans.

#### Related reports

In this work the authors use Rose-Bengal plate test (RBPT) to finalize the diagnosis of brucellosis. After reviewing the clinical and symptomatic aspects, the diagnosis is finalized by the intersection of clinical data with the Rose-Bengal plate test.

#### Innovations & breakthroughs

Brucellosis is a major zoonosis and in this work the authors demonstrated the use of rapid tests to screen for

possible infections. The addition of clinical information with serological data acquired by the Rose–Bengal plate test confirms the *Brucella* infection in humans.

### Applications

The use of serological tests, as Rose–Bengal plate test, to screen positive cases of *Brucella* makes the procedure faster and cheaper, allowing greater agility in identifying and initiating treatment.

### Peer review

This is an interesting work, where the authors report on human infection by *B. melitensis* which is helpful for clinical diagnosis and treatment.

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