Int J Clin Exp Med 2018;11(3):2709-2716
www.ijcem.com /ISSN:1940-5901/IJCEM0064835

Case Report

Eating goat's placenta and brucellosis caused by *Brucella melitensis*

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Received June 13, 2017; Accepted January 20, 2018; Epub March 15, 2018; Published March 30, 2018

Abstract: As zoonosis, brucellosis remains as a significant public health concern even in traditional low-risk areas. Pregnant women are susceptible to brucellosis. Here we present a case of pregnant-related brucellosis infected by eating goat's placenta. We showed its laboratory findings, and then review the literatures about pregnant-related brucellosis, laboratory findings of brucellosis and eating habits with *Brucella* infection. We also discuss the prevalence of brucellosis in low-risk southern China and the challenge we faced.

Keywords: *Brucella*, brucellosis, zoonosis, abortion

Introduction

Brucellosis, caused by infection with G^- intracellular coccobacilli [1], is the most widespread zoonosis all around the world. Thought progress in preventing the disease in many countries had been achieved, about 500,000 new cases are reported annually worldwide [1, 2], leading to great healthcare burden especially in developing countries. *Brucella melitensis (B. melitensis)* contributes to most of the brucellosis and has high risk of relapse [1, 3-5].

Both genders affect by brucellosis equally, but special attentions should be given to pregnant woman due to their damaged immunological system. *Brucella* infection leads to high abortion rate among animals [6]. Previously, the role of the bacteria in the abortion of human was controversy [6], but now increasing evidence demonstrates that *Brucella* infection may lead to devastating obstetric outcome, ranging from preterm labor to intrauterine fetal death [7-9]. The abortion rate of pregnant woman significantly increases and appropriate treatment could reduce brucellosis-related devastating obstetric outcome [8-10].

Close contact with infected animals or consumption of infectious animal products is the main pathway human being get infection of Brucella. Raw milk drinking is reported in most of patients of human brucellosis [8, 9, 11]. Here, we report a pregnant woman getting brucellosis due to the consumption of goat's placenta. We present the laboratory findings of patient and discuss their diagnostic value. We discuss relationship between peculiar dietary habit and *Brucella* spp. Infection. And in the end, we review the literature about prevalence of brucellosis in China, especially in low-risk urban settings, and discuss the challenge we face in controlling brucellosis (**Table 1**).

Material and methods

Data collection and analysis

The clinical data of the patient were obtained via reviewing the paper and electronic records during hospitalization, the results of white blood cell, red blood cell, Hb, hsCRP and PCT were obtained in our laboratory system. The microbiology data were obtained from the microbiology database. The history of animal contact and eating habits were obtained via inquiring the patient herself or her husband. All the procedures were conducted according to the guideline of national/hospital Ethics Committee of medical and with informed consent of the patient.

Table 1. Challenges we faced in preventing brucellosis

Region	Risk factors or challenges	References
National wide	Frequent market circulation, lack of disease quarantine	[15, 26-28]
Traditional Low-risk Region	Frequent market circulation, lack of disease quarantine, lack of experience	[15, 19, 20, 25, 29]
Guangdong province	Frequent market circulation, lack of disease quarantine, lack of experience, special eating habits, population migration	[15, 19, 25]

DNA extraction and AMOS-PCR

The DNA of *Brucella* was extracted with a DNAsorb-AM nucleic acid extraction kit according to manufacturer's instruction. The primers for PCR are as followed: AMOS-(A1) 5'-g AC g AA Cgg AAT TTT TCC AAT CCC-3', AMOS-(S) 5'-g Cg Cgg TTT TCT g AA gg T TCA gg-3', AMOS-(A2) 5'-g Cg CAg Cg T Tg C gg CAATTg-3' , AMOS-(IS711) 5'-Tgc Cg A TCA CTT AAg gg C CTT CAT-3'. The following program was used for DNA amplification: 95°C for 4 min , followed by 40 cycles of 60°C for 90 seconds/72°C for 10 mins, and then a final 72°C for 10 mins.

Case presentation

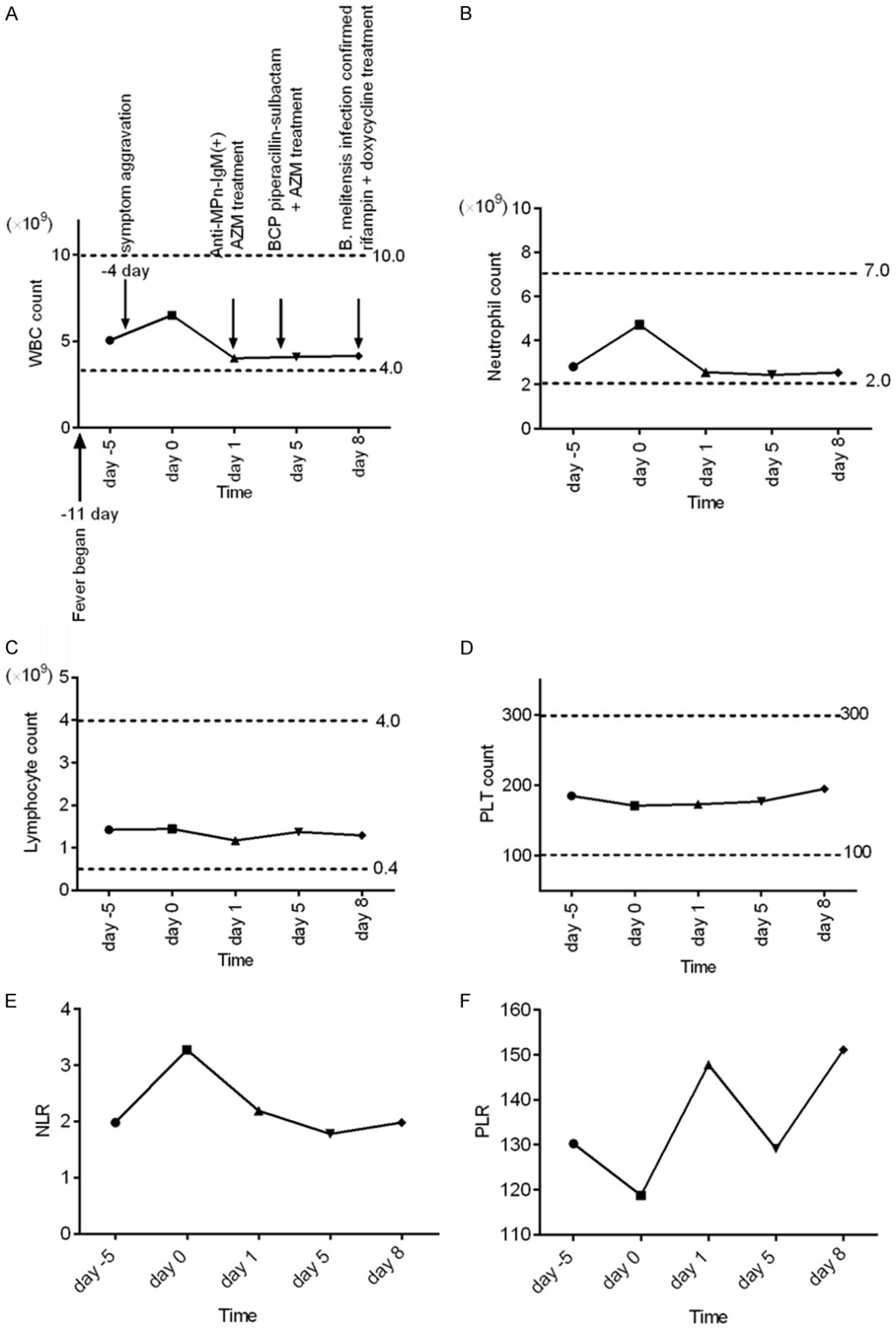
A 26-year old woman at her 12-gestational week experienced interval fever and shivering for 11 days, with highest body temperature of 39°C. Pharyngalgia, osphyalgia and malaise were also self-reported by the patient. She also had a history of intrauterine fetus death in January, 2016. She is an office clerk and lives in Zhaoqing city, Guangdong (south of China). On admission her temperature was 37°C, heart rate was 104/min, respiratory rate was 20/min. At the time of admission blood was drew for routine laboratory test and blood bacterial culture. All the results of the routine tests were within the reference level, except a slight decrease of RBC count and HGB. High sensitive C-response protein (hsCRP) and procalcitonin (PCT) were also detected, and hsCRP level was significantly increased while the PCT level was under medical determined level. The night on admission her body temperature went up to 38.5°C again, and interval fever was observed during hospitalization. Several blood samples were obtained for detection during hospitalization, the index of WBC, neutrophil, lymphocyte and platelet were with reference level and neutrophil to lymphocyte rate (NLR) or platelet to lymphocyte rate (PLR) were under determined level of system infection as the reference report (**Figure 1**). In contrast to WBC, a slight anemia was found (**Figure 2**). In her hospitalization, the hsCRP level was always beyond the determined level while PCT level was under the determined level (**Figure 3**). Two sets of blood culture performed at the time of admission revealed the presence of small gram-negative cocobacilli (**Figure 4**). The bacteria were then identified by Vitek2 Compact system with Vitek2 GN card. Main biochemical reactions of the bacteria were as follow: oxidase (+), catalase (+), urease production (+, <5 mins), hydrogen sulfide (-), D-glucose (-), D-maltose (-), D-mannose (-) and D-mannitol (-). The G^- cocobacilli was finally identified as *Brucella melitesi*s. On inquest, the patient denied directly contact with animals, like sheep, cow, and pigs and so on, but she admitted consumption of goat's placenta twice 2 months ago, and she had gingivitis at that time. To further validate the bacteria is indeed *Brucella*, blood sample was sent to The Center of Disease Control and Prevention of Guangdong province for serology and the isolates were sent to Sun Yat-sen University for molecular detection. Serology for *Brucella* was reported positive with a titer of 1:1280. AMOS-PCR (Abortus Melitensis Ovis Suis-PCR) was also used to identify the bacteria and the results confirmed the isolates were *Brucella* (**Figure 5**).

At the time of identification of *Brucella*, antimicrobial therapy with doxycycline (1 g bid) and rifampin (6 g qd) was initiated. Times of fever reduced and the level of hsCRP gradually decreased after antimicrobial therapy initiation. The patient was discharged to complete a 60-day course of antimicrobial therapy. A routine detection of the blood is conducted every other week post discharge to evaluate the treatment effect.

Discussion

Brucella infection in pregnant woman

High rate of adverse pregnant outcome was observed among animals with brucellosis. The role of *Brucella* infection in the pregnant out-


A
(×10^9)
WBC count
10
5
0
10.0
4.0
-4 day
symptom aggravation
Anti-MPn-IgM(+)
AZM treatment
BCP piperacillin-sulbactam
+ AZM treatment
B. melitensis infection confirmed
rifampin + doxycycline treatment
day -5
day 0
day 1
day 5
day 8
Time
-11 day
Fever began
B
(×10^9)
Neutrophil count
10
8
6
4
2
0
7.0
2.0
day -5
day 0
day 1
day 5
day 8
Time
C
(×10^9)
Lymphocyte count
5
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day 0
day 1
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Time
D
PLT count
300
200
100
300
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day -5
day 0
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day 5
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day -5
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day 1
day 5
day 8
Time
F
PLR
160
150
140
130
120
110
day -5
day 0
day 1
day 5
day 8
Time

Figure 1. The results of routine blood detection of the patient during hospitalization. (A-D) The number of white blood cell (WBC), neutrophil, lymphocyte and platelet during hospitalization was presented. Day 0 represents the time the patient be admitted, - represents before admission. (E and F) The ratio of neutrophil to lymphocyte (NLR) and platelet to lymphocyte (PLR) was calculated. Interval between imaginary lines represents reference value of respective indicator. MPn: mycoplasma pneumonia; AZM: azithromycin; BCP: blood culture positive.

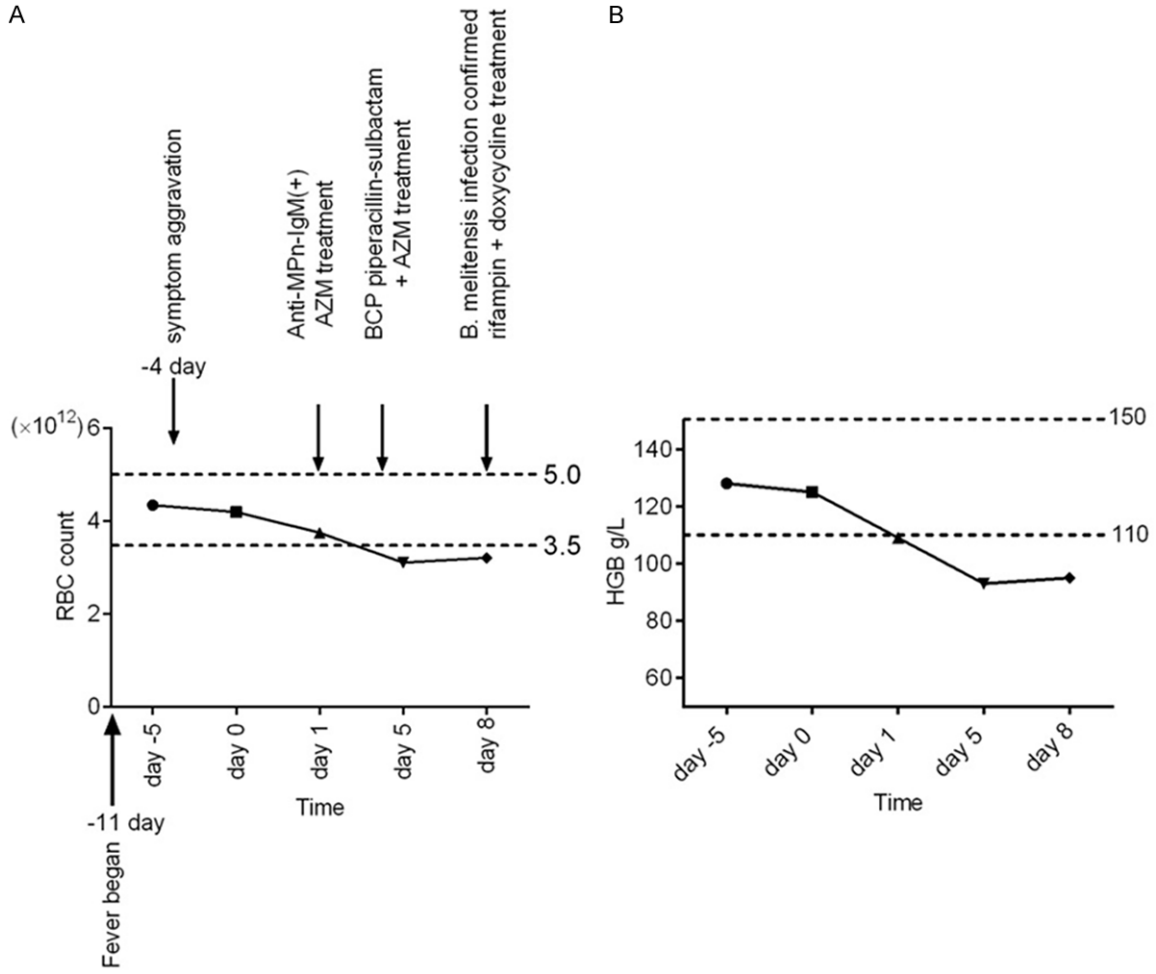

Figure 2. The results of red blood cell count and hemoglobin detection. The number of red blood cell (RBC) and the concentration of hemoglobin (HGB) during hospitalization were presented. Interval between imaginary lines represents reference value of respective indicator. MPn: mycoplasma pneumonia; AZM: azithromycin; BCP: blood culture positive.

come of human is controversial for a period [6, 12, 13]. More and more evidence demonstrates that *Brucella* infection leads to more adverse pregnant outcomes than other bacterial infection [8, 9, 14]. The incidence rate of brucellosis among pregnant women varies between 6%-17% in different studies [8, 10], and to our knowledge no such data could be obtained among pregnant women in China. Our case presented here is a pregnant-related brucellosis, and in review of literature we found a pregnant-related brucellosis has been reported elsewhere in China [15]. A retrospective study should be performed to better understand the incidence of pregnant-related brucellosis in China.

Adverse obstetric outcomes correlated with *Brucella* infection include abortion, preterm labor, intrauterine fetal death [8, 9]. The causes of spontaneous abortion and fetal death are not well known, and bacteremia, fever and toxemia are postulated mechanisms [16]. More than 40% of *Brucella* infecting pregnant woman

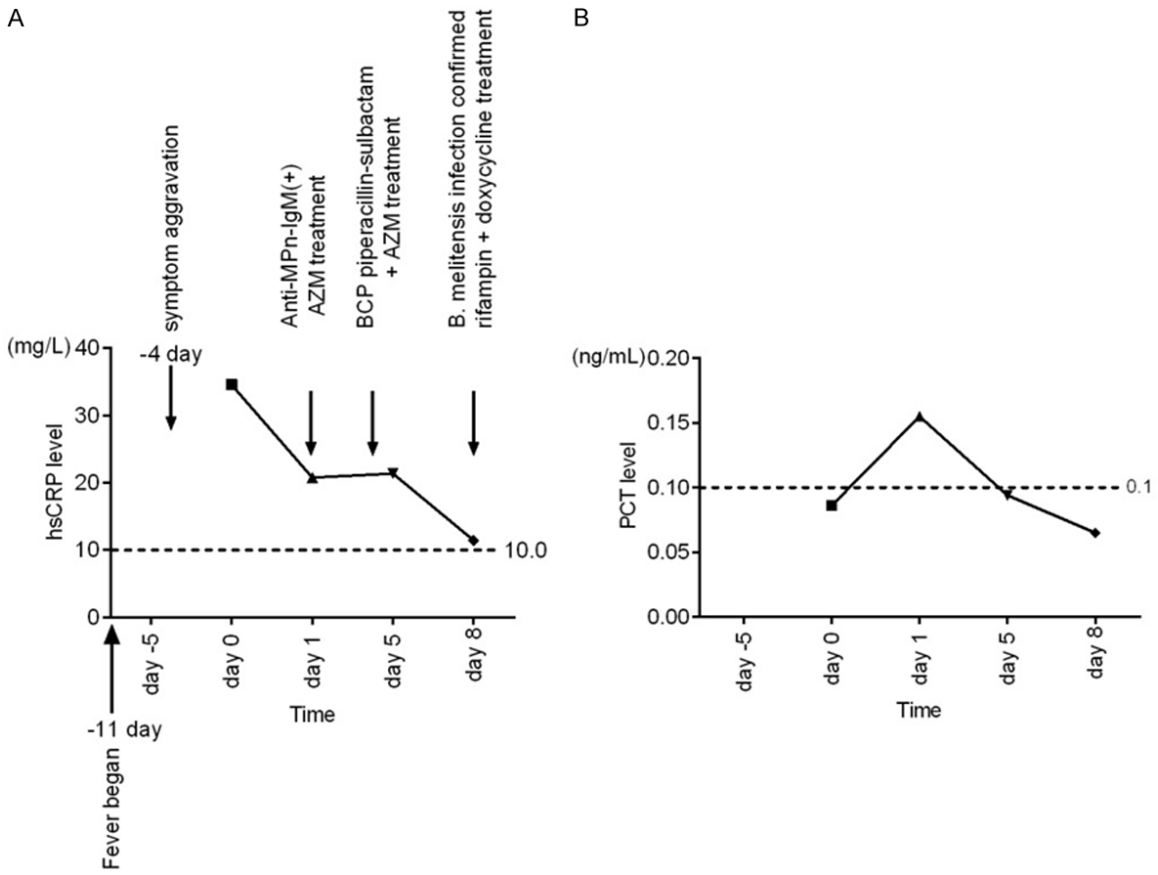

Figure 3. The results of hsCRP and PCT detection. The serum level of high sensitive C-response protein (hsCRP) and procalcitonin (PCT) was presented. Imaginary line represents reference level. MPn: mycoplasma pneumonia; AZM: azithromycin; BCP: blood culture positive.

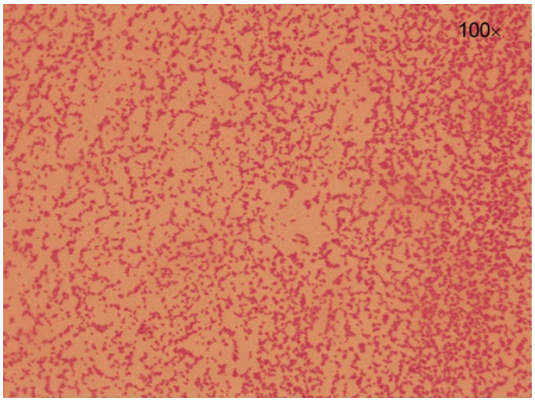

Figure 4. Gram staining of isolated bacteria. Positive blood culture material was transferred to sheep blood agar for bacteria separation. 48 hours later, the growing bacteria were Gram stained.

had adverse outcomes [8, 9]. In subacute *Brucella* infection with mild clinical presentation abortion is more common, and intrauterine fetal death is the main devastating pregnant outcomes of acute *Brucella* infection [8, 17]. Our cases reported a history of intrauterine fetal death; we did not know whether she got brucellosis at that time due to the lack of laboratory recording. Considering the habits of consumption of goat or sheep placenta in the region she live in and the undulant fever she experienced that time, *Brucella* infection may be top1 caused of the fetal death.

Blood cell count, hsCRP and PCT in brucellosis diagnose

Blood bacterial culture and serological method are the most important method in diagnosis of brucellosis, but due to the requirement of special equipment of blood culture and slow-growth of *Brucella* and relative high cost of serological method, new indicators for brucellosis diagnose is needed.

WBC and neutrophil count are significant increase in the case of bacterial infection, especially in those with bacteremia. But in our case as well as the cases reported by the oth-

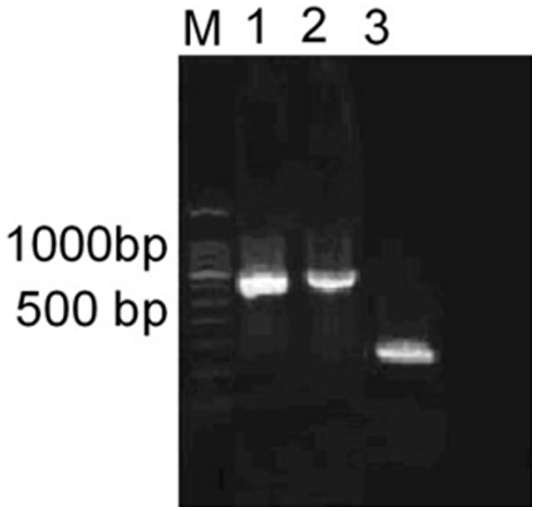

Figure 5. Gel electrophoresis of the PCR products. The PCR products were separated by gel electrophoresis. M: Marker. 1: Reference strain of Brucella melitensis. 2: Our isolate. 3: Negative control.

ers, the WBC and neutrophil did not significantly change [18-20]. Only ~10% of cases experienced leukocytosis [9]. In contrast to constant of WBC, mild anemia is very common among brucellosis patients [8, 9, 15, 19, 20]. In our case, mild anemia was seen during hospitalization. Several studies found that NLR and PLR could be indirect inflammation factor of brucellosis [21], but in our case these two factor remained constant. Both PCT and CRP are acute phase proteins and increased in bacterial infection. What's more, PCT is a promising maker for system bacterial infection and can be used to support clinical decisions regarding the initiation or discontinuation of antibiotic therapy [22]. In most of brucellosis cases reported, the level of CRP was significant increased and gradually reduced to reference level along with the antimicrobial used [19, 20, 23]. The change of CRP in our case was in accordance with references. The studies about the value of PCT in brucellosis diagnose is rare. In our case, we found that PCT level remained constant during hospitalization.

Together, for those with undulant fever in combined with mild anemia and increasing CRP level should be considered of brucellosis. The history of animal contact or animal product consumption should be inquired. Blood culture and serological test should be conducted.

Special eating habits and Brucella infection

As a zoonosis, brucellosis affects human mainly via direct contact with infected animals. But as other infectious diseases brucellosis may sometimes be acquired via eating. In epidemic regions of brucellosis, raw milk or non-pasteurized dairy products consumption is very common [8, 9, 17, 24]. In the study of Mertihan and Gustavo [8, 9], they found more than 90% of brucellosis patients had a history of non-pasteurized dairy. Van herby cheese (a kind of cheese manufactured mainly in rural areas by villagers) is one of popular dessert in Turkey, and consumption of Van herby cheese is a risk factor of brucellosis [8, 9, 17].

In our case, we found that the patient had a history of eating goat's placenta, and it's the most possible way she got *Brucella* infection. In traditional Chinese medicine goat or sheep placenta is considered nutritious, thus Placenta consumption is popular in China especially in Guangdong province, south of China. In a survey of the prevalence of brucellosis from 2005 to 2010 in Guangdong province, more than 20% of the cases had a history of goat placenta eating [25], and it's the only risk factor of these patients. In the report of Chen [15], a pregnant woman with history of goat placenta eating was infected with *Brucella*, and her twins baby were also infected. Placenta consumption may be one of the most important pathways of *Brucella* infection in Guangdong.

Prevalence of brucellosis in low-risk urban region in south of China and the challenge we faced

The first case of brucellosis in China was reported in 1905, and the incidence of brucellosis remained at low level from 1980s, but in recent year incidences of human brucellosis rapidly increased [26]. Traditionally, north or northwest of China is epidemic region of brucellosis; Inner Mongolia, Shanxi, Heilongjiang and Hebei rank top forth of brucellosis incidence in China [27, 28]. Recently, the affected areas expanded rapidly, and the cases reported in southern or eastern coastal provinces significantly increased [27-29]. As one of the most developed regions in China, the incidence of brucellosis was lowest in Guangdong province, <0.01 cases/100,000 population [25]. But the cases

of brucellosis sharply increasedduring recent decade [15, 19, 25]. Guangzhou city and Shenzhen City reported most of cases (>70%), and *B. melitensis* infection accounts for most the cases [25].

In northern or northwestern of China, occupational exposure is the main risk factor of human brucellosis. But the risk factor of brucellosis in southern of China varied, and occupational exposure account for only small fractions of the cases. Special eating habits, like goat placenta eating habits in Guangdong, may account for heavy proportion of the cases [15, 19, 25]. As open and well developed cities, Guangzhou and Shenzhen attract many people from northern or northwestern of China as well as people from African and Indian subcontinent. All these regions have high incidence of brucellosis [6, 27, 28, 30], which increases the risk of input brucellosis in these two cities. Increased population migration (mainly from northern to southern) and increased livestock trading may promote the increase of brucellosis. Rapid development of expressage made e-trade of animal products (milk and meat, et. al., mainly directly from farmer to customer), easy, but made quarantine difficult or missing. It may be one of latent risk factor of brucellosis in low-risk urban area in China, which should be paid attentions.

Unlike the doctors in brucellosis epidemic regions, the clinicians in low-risk urban region may overlook the diagnosis of brucellosis due to the lack of specific signs or symptoms. Thus for those with interval fever without other symptoms or signs, the history of animal contact or animal products consumption inquire and blood culture or serological detection of *Brucella* antibody may help the diagnosis a or treatment of the patients.

Conclusion

Diagnosis of brucellosis is still challenge. For those with only interval fever in combined with mild anemia and increased CRP level, differential diagnosis of brucellosis should be considered. Obtain of clinical history animal contact and animal product consumption is valuable.

Acknowledgements

We thank Dr. Yu Zheng in Sun Yat-sen University for the assistance in molecular identification of the isolates.

Disclosure of conflict of interest

None.

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