# Comparison of B.melitensis and B.abortus Bacteremias with Respect to Diagnostic Laboratory Tests

# Laboratuvar Tanı Testleri Açısından B.melitensis ve B.abortus Bakteriyemilerinin Kıyaslanması

B.melitensis, B.abortus, Bakteriyemi, Tanı / B.melitensis, B.abortus, Bacteremias, Diagnosis

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**Özet**

Amaç: Bruselloz, Brucella melitensis ve Brucella abortus türlerinin sıklıkla etken olduğu bir enfeksiyon hastalığıdır. Bu çalışmada B. melitensis ve B. abortus'un etken olduğu bakteriyemilerin, rutinde sıklıkla yapılan tanı testlerine dayanarak (serolojik test ve kan kültürü pozitifliği), birbirinden ayırt edilebilmesi amaçlandı. Gereç ve Yöntem: Bu çalışmada, Ocak 2010 ve Nisan 2014 tarih aralığında kan kültürlerinden Brucella sp. izole edilen 42 hasta çalışma kapsamına alındı. Bruselloz şüphesi olan hastalardan 8-10 ml kan kültürü için örnek alındı ve BACTEC plus/Aerobic F kültür şişelerine aktarıldı. Alınan kan kültürü örnekleri BACTEC 9240 cihazında (BD Diagnostic, Maryland, USA) 21 gün süre ile inkübe edildi. 42 hastanın her birinin, kan kültürü ile eş zamanlı alınan kan örneğinden ayrılan serumlarda, Rose Bengal ve Standart Tüp Aglütinasyon (STA) (Spinreact, Spain) testleri çalışıldı. Bulgular: Akut bruselloz tanısı konulan hastalara ait en az 2 adet alınan kan kültürü örneklerinin, cihazda pozitif sinyal verme süreleri B. melitensis ve B. abortus türleri için karşılaştırıldığında, anlamlı bir fark olmadığı görüldü (saat için p=0.850; gün için p=0.696). Cihazda pozitif sinyalin en erken 2. günde, 44. saatte; en uzun 6.günde 123.saatte olduğu, cihazda ortalama pozitif sinyal verme süreleri açısından iki tür arasında anlamlı fark olmadığı saptandı. Tartışma: Bu çalışmada B. melitensis (n:22) ve B. abortus'un (n:20) etken olduğu bakteriyemiler arasında yaş, cinsiyet, kan kültürü pozitiflik zamanı ve STA test titre düzeyleri açısından anlamlı bir fark olmadığı görüldü.

**Anahtar Kelimeler**

B.melitensis; B.abortus; Bakteriyemi; Tanı

**Abstract**

Aim: Brucellosis are most commonly caused by the Brucella species Brucella melitensis and Brucella abortus. This study was aimed to determine the differences in the routine diagnostic tests (serological tests and blood culture positivity) that differentiate bacteremias caused by B. melitensis and B. abortus. Material and Method: This study included a total of 42 patients from whose blood cultures Brucella sp. were isolated between January 2010 and April 2014. A 8-10 ml blood sample was put into BACTEC plus/Aerobic F culture bottles after being drawn from patients (n:42) with suspected brucellosis. The obtained samples were incubated in BACTEC 9240 device (BD Diagnostic, Maryland, USA) for 21 days. Sera of the blood samples taken simultaneously with the blood culture were studied with the Rose Bengal and Standard Tube Agglutination (STA) tests. Results: In patients with acute brucellosis, B. melitensis and B. abortus species showed no significant differences with respect to time to positive signal in blood cultures (for hours p=0.850; for days p=0.696) and the mean time to positivity. The earliest signal in the device was delivered at day 2., 44th hour and the latest at day 6., 123rd hour. No significant difference was noted between the two species with respect to the mean time to positivity. Discussion: This study did not show any significant differences between B. melitensis (n=22) and B. abortus (n=20) bacteremias with respect to age, sex, time to blood culture positivity, and STA test titer level.

**Keywords**

B.melitensis; B.abortus; Bacteremias; Diagnosis

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## Introduction

Brucellosis, caused by the Brucella species, is one of the most significant zoonotic diseases causing both human and animal infections. Brucellosis is endemic to densely populated developing countries of Asia, Middle East, and Latin America.

Humans are usually infected by directly contacting infected animal tissues, ingesting unpasteurized animal products, or breathing bacteria proliferated in laboratory media. Research has shown that although different Brucella species have been shown to infect domestic animals (cows, sheep, goats, pigs, camels, reindeers, dogs), humans are usually infected by Brucella melitensis, Brucella abortus, Brucella suis, and rarely Brucella canis species [1].

Brucella species are able to escape body defense mechanisms and live intracellularly; thus they cause prolonged, relapsing-remitting infections with a high morbidity. They cause systemic infections that potentially involve any organ or system and are characterized by serious bacteremia. Brucellosis can be diagnosed by isolating the microorganism from blood, body fluids, or infected tissues. Additionally, seropositivity can be demonstrated by a titer of 1:160 or greater in the Standard Tube Agglutination Test (STA), a titer of 1:320 or greater in the Coombs' test, or seroconversion that is supported by clinical signs [2].

Brucella infections are most commonly caused by the Brucella species B. melitensis and B. abortus [3, 4]. B. melitensis is more common in our country [5]. Distinguishing Brucella species from each other is not only important for finding the source of infection, but also for determining clinical and epidemiological characteristics. It has been reported that the infections caused by B. melitensis and B. suis have a more virulent course than those caused by B. abortus and B. canis [5-7].

Identification of these two species clinically and epidemiologically is important. Identification of the Brucella species requires a proper isolation process and a series of biochemical or serological tests (CO2 requirement for growth, urease activity, H2S formation, sensitivity to basic fuchsin and thionin stain, and Tbilisi phage).

In the present study, acute brucellosis patients having B. melitensis and B. abortus bacteremia were compared with each other with respect to age, sex, serological test (STA) titer results, and the time to positive signal in BACTEC 9240 device. Symptoms and signs of cases of acute brucellosis were defined as less than 2 months. It was aimed to differentiate bacteremias caused by B. melitensis and B. abortus by using the differences in the routine diagnostic tests (serological tests and blood culture positivity time) caused by each of the two bacteria to be used in future in cases where the suspected species cannot be isolated or identified. If we find differences between these two types, can be distinguished by using serologic tests and blood cultures positivity time results in the laboratory.

## Material and Method

Patient population: This study included a total of 42 patients from whose blood cultures Brucella sp. were isolated between January 2010 and April 2014. A total of 82 patients suspected brucellosis, 42 of them (51.2%) were found positive blood culture. All of 42 patients were diagnosed with acute brucellosis and had bacterial growth in at least two blood cultures.

Blood culture: A 8-10 ml blood sample was drawn from each patient with suspected Brucellosis and then it was put into BACTEC plus/Aerobic F culture. The obtained samples were incubated in BACTEC 9240 device (BD Diagnostic, Maryland, USA) for 21 days. From the bottles that delivered a positive signal, blood samples were drawn for gram staining and subculturing in 5% sheep blood and chocolate agar media and they were incubated under aerobic and 5 to 10% CO2 conditions.

Bacteria identification: To identify proliferating bacteria, gram staining, urease activity, H2S production, and sensitivity to basic fuchsin and thionin (20 and 40 µg/ml) stains were studied. In addition, tests were performed with polyvalent antiserum specific to Brucella species (B. abortus, B. suis, B. melitensis) and lam agglutination test with Brucella abortus and Brucella melitensis monovalent antisera (RSHM antisera, Ministry of Health, Turkish Public Health Institution, Bacterial Zoonosis Research and Reference Laboratory). The species were identified according to the scheme for identification of Brucella species [8].

Serological tests: Sera of the blood samples taken simultaneously with the blood culture samples were studied with the Rose Bengal and STA (Spinreact, Spain) tests, as recommended by the manufacturer. The sera were diluted at a range of 1:20 to 1:1280 for STA.

Statistical Analysis: SPSS 17.0 software package (Version 17.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Sex, age group, serological test results, and time to positive signal in blood culture samples (hour/day) were compared in 42 patients with acute brucellosis caused by B. melitensis and B. abortus species isolated from blood cultures. The categorical data were presented as number and percentage and the continuous variables as median and minimum-maximum. The Fisher Test was used to compare the categorical variables; the non-normally distributed continuous variables were analyzed with the Mann Whitney-U test. A p value of less than 0.05 was considered statistically significant.

## Results

Of 42 samples, 22 contained B. melitensis and 20 contained B. abortus. The age range was 5 to 82 years. There was no significant difference between the species with respect to age distribution (p=0.130). The numbers of both sexes were equal in the study population (each having 21 subjects). The demographic characteristics of the patients were summarized in Table 1.

Table 1. Demographics characteristics of bacteraemic patients

| Gender | Brucella spp. | | Total |
|---|---|---|---|
| | B.melitensis (n) | B.abortus (n) | |
| Female | 9 | 12 | 21 |
| | 40% | 60% | 50% |
| Male | 13 | 8 | 21 |
| | 59% | 40% | 50% |
| Total | 22 | 20 | 42 |
| | 100% | 100% | 100% |

The analysis of the anti-brucella antibody levels determined by the STA test revealed that three patients were negative and the maximum titer was 1:1280. No dilution was carried out beyond a titer of 1:1280.

B. melitensis and B. abortus species had no significant differences with regard to the time to positive signal in at least two blood cultures (for hours p=0.850; for days p=0.696). The earliest signal in the device was delivered on the 2nd day, 44th hour and the latest on the 6th day, 123rd hour. The two species had no significant differences in the mean time to positivity. The analysis of the seasonal distribution of the causative species showed that B. melitensis (n:8) and B. abortus (n:8) infections were most prevalent in Summer. The two species did not differ significantly with respect to the month of bacteremia (p=0.849).

## Discussion

As in the rest of the developing world, Brucellosis is still endemic to Turkey. Based on the Turkish Ministry of Health Statistics 2005 data, the morbidity rate of brucellosis is 20,32/100.000 and the number of cases was reported to be 14.644 [9].

No sex predilection has been reported among cases infected by B. melitensis or B. abortus [4, 7]. Likewise, our study did not demonstrate any significant differences between the two genders with respect to infection, although B. melitensis was isolated more commonly in men (n=13) and B. abortus was more common in women (n=12) (Table 1).

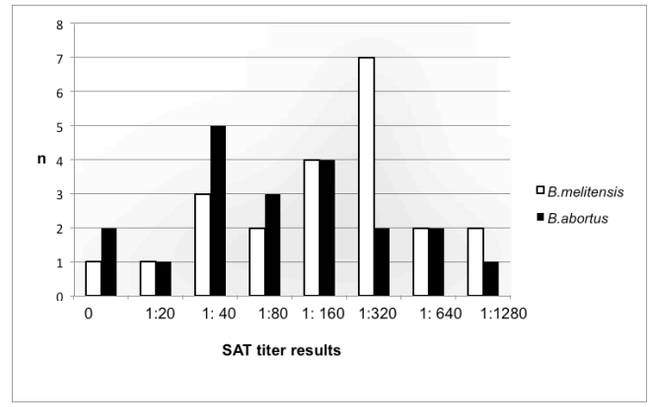
Isolation of the causative agent from blood cultures, bone marrow, and other body fluids is the gold standard for diagnosis of brucellosis [10]. However, bacterial isolation may sometimes be problematic, especially in patients with chronic brucellosis. The overall positivity rate of blood cultures ranges between 15% and 70% [2]. It has been reported that 90% of the Brucella species are isolated within 7 days of culturing with the use of the BACTEC blood culture technique [11]. Isolation rates of 82.4% (12) and 92.7% [13] have been reported after a 5-day-incubation period. In this study, approximately 93% of the acute brucellosis cases could be detected within a 5-day incubation. A positive signal for bacteria was delivered at 6th day in 3 (7%) patients. Extending the incubation period is usually required in order to diagnose all of the cases. In our study, all of 42 acute brucellosis cases could be detected with the BACTEC 9240 device within the first 7 days.

The time to blood culture positivity has been reported 2.9 days (69.87 hours) by Durmaz et al. [14] ; 3,5 days by Yakupsky et al. [15] ; and 2,8 days by Baysallar et al. [16]. There was, however, only one study that specifically studied and found no significant difference between the difference of time to a positive signal in blood cultures between B. melitensis and B. abortus species [13].

An average time to a positive signal of 65.50 hours/3.70 days for B. abortus and 66.00 hours/3.55 days for B. melitensis (p=0,696) suggested that it was not useful in distinguishing the two species from each other.

In the absence of isolation of any causative bacteria, the diagnosis of the disease is made by serological tests (STA, Coombs' test, Brucellacapt test, ELISA methods), detection of the anti-brucella antibodies, or molecular techniques [17]. We found no significant differences between the two species with respect to the titers of the STA test. As shown in Figure 1, however, blood culture proliferation most commonly occurred at a titer of 1:320 for B. melitensis (n=7) and 1:40 for the B. abortus (n=5). Despite being low in number to draw a solid conclusion, the B. melitensis cases, as compared to B. abortus cases, had higher STA titer determined simultaneously with blood culture positivity (Figure 1). This difference may be useful to differentiate the two species. It has been reported that a STA test titer of >1:160 had a sensitivity of 92% for the diagnosis of acute brucellosis [2]. In this study, 24 cases had a titer equal to or greater than 1:160. It was demonstrated that the STA test was diagnostic in 57% (n: 24) of the cases. Eleven (61%) of the 18 cases with a titer less than 1:160 had B. abortus bacteremia. This suggests that in cases with a STA titer less than 1:160 a blood culture should be sent in an attempt to hasten the diagnosis. It was noted that the majority of such cases were B. abortus bacteremia.

Figure 1. Comparison of standart tube agglutination test results with Brucella species in 42 patients




In conclusion, this study did not show any significant differences between B. melitensis (n=22) and B. abortus (n= 20) bacteremia with respect to age, sex, time to blood culture positivity, and the titer of the STA test. The available tests to distinguish species will continue to exist until novel diagnostic methods are introduced.

### *Competing interests*

The authors declare that they have no competing interests.

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