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Diagnosis of brucellosis by use of BACTEC blood culture and confirmation by PCR

Maleknejad, P.[1]*, Peeri-DoGaheh, H.[2], AmirZargar, A. A.[3], Jafari, S.[4], Fatollahzadeh, B.[1]

[1]*Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran-Iran.*

[2]*School of Medicine, Ardebil University of Medical Sciences, Ardebil-Iran.*

[3]*Department of Immunology, School of Medicine, Tehran University of Medical Sciences Tehran-Iran.*

[4]*Department of Infectious Diseases, School of Medicine, Tehran University of Medical Sciences, Tehran-Iran.*

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Abstract: **Brucellosis is one of the most common zoonotic diseases in Iran. Growth of *Brucella* is slow and blood cultures of these bacteria are time-consuming via classical methods. We try to evaluate BACTEC 9120 system capacity in order to detect of bacteremia due to *Brucella spp* and to confirm isolated bacteria by PCR. Blood culture sample of 102 suspected patients evaluated by BACTEC 9120 system. They were subcultured when the machine detected their growth; if not; blind subcultures were performed on days 7, 14, 21 and 28. Forty-one of 102 suspected patients showed bactermia. Isolation rate of *Brucella* was 40.2%. All patients were detected by BACTEC 9120 system. All positive blood culture was detected via BACTEC 9120 and blind subcultures. No positive blood culture bottles were missed by the system. Our data obtained by using the BACTEC 9120 system indicates a more rapid detection of *Brucella* than conventional methods.**

Key words: brucellosis, BACTEC system, blood culture.

Introduction

Brucellosis is a widespread zoonosis caused by members of the genus *Brucella*. The disease is accidentally transmitted to humans during occupational contact with infected animals or by consumption of contaminated animal products (1). Brucellosis remains an endemic disease in many regions of the world, and is more prevalent in developing countries, particularly in west parts of Asia, India, Middle East, Southern European, and Latin American countries. Human brucellosis is endemic in all parts of Iran. The numbers of patients recorded in 1988 were 71, 051 (132.4 per 100, 000) (2). The laboratory confirmation of human brucellosis is based either on isolation of *Brucella* from blood cultures or detection of specific antibodies. Blood cultures which provide the best yield for microbiological diagnosis, with sensitivity ranging from 53 to 90%, depend on the disease stage, *Brucella* species, culture medium, quantity of circulating bacteria, and employed culture technique. Serology plays a major role in cases where the disease cannot be detected by culture. Serological tests are rapid and easy to perform (3), but their interpretation is often difficult, particularly during the early stage of the disease (4). Despite recent developed techniques, like nucleic acid probes, PCR, and other molecular techniques for Microbiological diagnosis, blood cultures still remain the most practical and reliable method in the diagnosis of bloodstream infections (5). In endemic areas culture is required for a definitive diagnosis because the symptoms of brucellosis are non-specific, and the interpretation of agglutinating antibody titer can be confounded by

* **Corresponding author's email: maleknej@sina. tums. ac. ir, Tel: 021- 88955810, Fax: 021-88953001**

persistently elevated titer in persons without active disease who have been repeatedly exposed to infected animals (6). Before detection of growth, blood culture which is done by the classical Castaneda method can take up to 35 days of incubation (7). Modern automatic blood culture systems have reduced detection times of microorganisms which produce bacteremia, allowing incubation times to be reduced to 7 days or less (8). In this study, we aimed to find out the rate of isolation of *Brucella spp* in blood by use of the BACTEC 9120 system.

Materials and Methods

From 2002 to 2004, 102 blood culture samples of suspected patients were admitted to microbiology laboratory of Tehran University of Medical Sciences. Suspicions of brucellosis were serologically confirmed with Rose Bengal antigen test. All the patients were adults, and had high titer anti-*Brucella* antibodies at the time of diagnosis. High titer was defined as a titer of ≥ 1/160 by the standard tube agglutination (STA) method. Only one blood culture was processed per patient. Blood for culture was drawn by the staff physicians, and between 5 to 7 ml of blood from patients was inoculated in each of standard aerobic/F BACTEC bottle at the patient's bedside. Upon receipt in the microbiology laboratory, BACTEC Aerobic/F bottles were loaded into the instrument in the computer-assigned position and incubated for 7 days with continuous agitation. The BACTEC 9120 instrument was observed at 4-h intervals for positive signals. Whenever a positive signal occurred, the bottle was removed from the instrument to be Gram stained and subcultured. The BACTEC 9120 instrument monitors increases in CO_2 concentration produced by growing microorganisms by means of a fluorescent sensor are located in the bottom of each bottle (9). The microscopic slid which was prepared from all cultures showed positive and negative growth index with gram method then they were subcultured on chocolate agar and incubation at 37°C in microaerophilic condition. The isolates of Gram-negative coccobacilli were identified by conventional tests (e. g., oxidase, catalase, production of H2S and urease) and also by an agglutination test using antisera. In addition, PCR were used for precise identification of isolated bacteria. A PCR assay with primers B4 (5'-TGG CTC GGT TGC CAA TAT CAA-3') and B5 (5'-CGC GCT TGC CTT TCA GGT CTG-3') (MWG-biotech, Germany) was used to detect *Brucella* DNA. These primers specifically amplify a 223-base pair fragment from the conserved region of the gene, which encodes an immunogenic membrane protein of 31 KDa of *Brucella* abortus, specific to the *Brucella* genus and present in all its biovars (Figure 1). At the end of the first week, bottles with negative growth index were kept for an additional 3 weeks and blind subcultures of samples from the blood culture bottles were performed on days 7, 14, 21, and 28. Cultures were considered negative for *Brucella* only after four weeks of incubation.

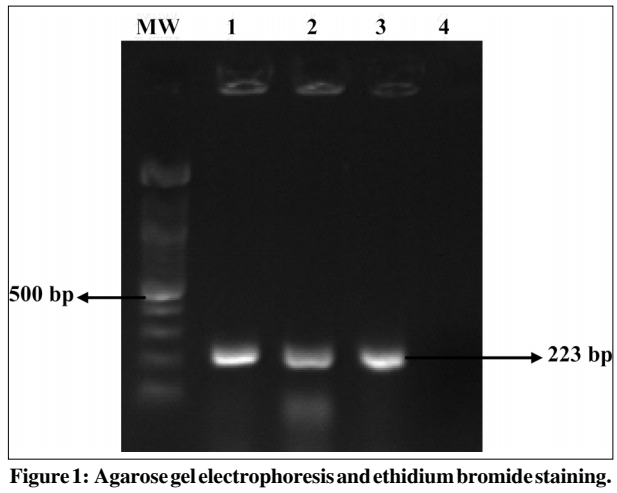

Figure 1: Agarose gel electrophoresis and ethidium bromide staining. Lane MW, DNA ladder; lane 1, 2, DNAs from two *Brucella* isolates; Lane 3, positive control (*B. abortus* DNA); lanes 4, Negative control.

Results

A total of 102 blood culture bottles were processed during the evaluation period: 41 of them (40.2%) gave a growth index positive (GIP) by the BACTEC system and no growth was detected by BACTEC system in 61 (59.8%) cases, and they were growth index negative (GIN). All blood culture bottles were subcultured on sheep blood agar, chocolate agar and MacConkey plates. Seeded media were incubated at 35°C in a 5% CO_2-enriched atmosphere and examined daily for 4 days. Gram

staining was performed in all BACTEC broths. Subculture of 41 (GIP) BACTEC broths on sheep blood and chocolate blood agar eventually yield *Brucella* organisms within two to three days. Thirty-nine BACTEC broths gave a visible colony within two days, but two isolates gave a visible colony after three days. All cultures positive with the BACTEC instrument were detected within a week and blind subculture of 61 (GIN) BACTEC broths on days 7, 14, 21 and 28 was negative. All bottles of positive blood culture were detected by the BACTEC 9120 instrument and by blind subcultures. Gram staining detected organisms in 26 of 41 (GIP) BACTEC broths. None of 61 (GIN) BACTEC broths for *Brucella* showed presence of Gram negative coccobacilli.

Discussion

In the last decade, automated blood culture system, based on continuous monitoring and colorimetric detection of CO_2 production of growing organisms has been introduced into clinical practice. Recent studies have demonstrated that this technology enables most clinical microbiology laboratories to detect genus *Brucella* within the 7-day routine incubation period (10). Yagupsky isolated 21 of 27 (78%) B. melitensis strains by using BACTEC NR 660 system and also by blind subcultures in less than 7 days (1). Ruiz *et al*., recovered 16 out of 17 (94%) isolates with BACTEC 9120 system (Becton Dickinson) during 7 days of incubation (8). Two different studies using BACTEC 9240 system reported that 93 and 100% of the isolates were recovered during 5 days (7, 10). No data on the performance of BACTEC blood culture system for detection of *Brucella spp* have been published from Iran and this is the first and the largest reported study. In our study, the isolation rate of blood cultures was 40.2% and in comparison with other studies (7, 10) it seemed to be a low rate. There are three possible explanations for the low efficiency in isolating *Brucella* from our blood culture. These factors which may be involved are one, or any combination of factors listed below:

1- It is possible that some of the suspected patients would be in chronic stage in which detection of *Brucella* is difficult. Serra and Vinas isolated *Brucella* from 92.5% of blood cultures of patients in acute phase, but only 8.3% of those from patients were in chronic phase (11).

2- The effect of consumption of antibiotics on participants, the same as the effect on suspected patients decreases the isolation rate of *Brucella* in blood culture.

3-Since seropositive status for brucellosis is common in endemic areas, and Iran is an endemic region for brucellosis, thus the patients might suffer from other chronic diseases and be seropositive for brucellosis (12).

We believe that if we had examined specimens from patients who had higher titers such as |"|1/320 or convalescence titers which are four times higher than acute phase against *Brucella*, the probability of recovering *Brucella* would have been improved. Our results suggest that a 7-day incubation period in the BACTEC system is sufficient for the detection of these slow-growing bacteria and for detection of *Brucella spp* by BACTEC system, therefore prolonged incubation time and periodic performance of subcultures are not required.

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تشخیص بروسلوز با استفاده از سیستم کشت خون BACTEC و تائید نتایج آن با PCR

پرویز مالک نژاد[۱]* هادی پیری دو گاهه[۲] علی اکبر امیرزرگر[۳] سیروس جعفری[۴] بهرام فتح اله زاده[۱]

[۱] گروه میکروبشناسی دانشکده پزشکی دانشگاه علوم پزشکی تهران، تهران - ایران.
[۲] دانشکده پزشکی دانشگاه علوم پزشکی اردبیل، اردبیل - ایران.
[۳] گروه ایمونولوژی دانشکده پزشکی دانشگاه علوم پزشکی تهران، تهران - ایران.
[۴] گروه بیماری های عفونی دانشکده پزشکی دانشگاه علوم پزشکی تهران، تهران - ایران.

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بروسلوز از جمله بیماری‌های شایع مشترک بین انسان و حیوان در ایران است که توسط جنس بروسلا به انسان سرایت می‌نماید. از آنجایی که در نواحی اندمیک جداسازی باکتری برای تشخیص قطعی ضروری است بر آن شدیم تا توانایی سیستم BACTEC 9120 را در ردیابی باکتریمی ناشی از بروسلا ارزیابی نموده و نتایج حاصله را نیز توسط PCR تائید نماییم. بدین ترتیب مطالعه مقطعی طراحی شد که طی آن از ۱۰۲ بیمار مشکوک به بروسلوز مقدار ۷ - ۵ میلی لیتر خون گرفته شد و به ویالهای کشت BACTEC aerobic/f تلقیح شد. ویالها در دستگاه BACTEC 9120 به مدت ۷ روز انکوبه شدند. از ویالهایی که توسط دستگاه مزبور مثبت اعلام شده بود کشت مجدد انجام شد. در مواردی که نمونه پس از ۷ روز توسط BACTEC منفی اعلام می‌شد، از آنها در دروزهای ۷، ۲۱، ۱۴ و ۲۸ کشت مجدد انجام شد. در ۴۱ (۴۰/۲ درصد) مورد از ۱۰۲ بیمار مشکوک به بروسلوز، باکتریمی توسط BACTEC ردیابی شد که تمامی آنها توسط PCR نیز تائید شدند. مواردی که در کشت مجدد مثبت شود ولی در سیستم BACTEC منفی گزارش شود مشاهده نشد. مطالعه ما نشان می‌دهد که استفاده از سیستم BACTEC نسبت به روش‌های متداول در ردیابی بروسلا روشی سریعتر می‌باشد.

واژه‌های کلیدی: بروسلا، بروسلوز، سیستم BACTEC، کشت خون.

*) نویسنده مسؤول: تلفن: ۸۸۹۵۵۸۱۰ - ۰۲۱ نمابر: ۸۸۹۵۳۰۰۱ - ۰۲۱ Email:maleknej@sinatums.ac.ir