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Detection of *Brucella melitensis* by BACTEC NR660 Blood Culture System

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Data on the performance of modern blood culture systems for the detection of *Brucella* spp. are insufficient. To evaluate the performance of the BACTEC NR660 blood culture system for the detection of *Brucella melitensis* within the routine 1-week blood culture protocol, a prospective 24-month study was conducted in an area endemic for *B. melitensis* in southern Israel. Blood samples obtained from patients with suspected brucellosis were monitored and blindly subcultured once per week for 4 weeks, and the fraction of blood cultures positive for *B. melitensis* detected by the BACTEC NR660 instrument within the first week was determined. During the study period, a total of 373 blood cultures were obtained from patients in whom brucellosis was suspected, and 27 (7.2%) of them, drawn from 21 different patients, were positive for *B. melitensis*. Twenty-one (78.8%) of these positive cultures were detected by the BACTEC instrument within 7 days, and six positive cultures were detected by subculture after 2 or 3 weeks of incubation. It is concluded that the BACTEC NR660 blood culture system detects the majority of *B. melitensis* isolates within the routine 1-week blood culture schedule. To maximize the recovery of the organism, however, prolonged incubation and periodic performance of blind subcultures are still required.

Brucellosis is a worldwide 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 (5). The diagnosis of brucellosis is established with certainty by isolation of the organism from blood and other normally sterile body fluids (4, 5). Because of the fastidious nature of *Brucella* spp., incubation of blood cultures for up to 30 days instead of the regular 5 to 7 days and performance of periodic blind subcultures have traditionally been recommended to maximize the isolation of the organism (2, 5). These recommendations are based on the experience accumulated in the past with the use of traditional blood culture methods, but information on the performance of modern blood culture techniques is scarce. To investigate the capability of the nonradiometric BACTEC blood culture system to detect *Brucella melitensis*, a prospective study was conducted in a medical center serving an area in southern Israel endemic for *B. melitensis*.

MATERIALS AND METHODS

Background. The Soroka Medical Center is a tertiary-care, 750-bed university hospital located in the city of Beer-Sheva in the Negev Desert area of southern Israel. The Soroka Medical Center serves a population of 320,000 inhabitants, two-fifths of whom are Bedouin Arabs and the rest are of Jewish origin. Brucellosis caused by *B. melitensis* is endemic in the region, and in 1988 the attack rate of the disease was as high as 52 per 100,000 inhabitants (1). Most of the diagnosed patients are Bedouins who maintain herds of unvaccinated animals in close proximity to the living quarters and who consume unpasteurized dairy products.

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Bacteriological methods. BACTEC 6NR (aerobic) and 7NR (anaerobic) blood culture bottles were available at all patient locations throughout the hospital. Blood for culture was drawn by the staff physicians, and blood culture bottles were inoculated with 3 to 5 ml of blood at the patient's bedside. Because *Brucella* spp. are strict aerobic organisms, only the methodology used for the aerobic medium will be described in detail.

(i) Routine blood cultures. Aerobic bottles were incubated at 35°C for a maximum of 7 days and were agitated during the first 24 h of incubation. The headspaces of the bottles were analyzed by the BACTEC machine twice per day on days 1 and 2 and once per day on days 3, 5, and 7. Criteria for positivity were a growth index (GI) of >25 and/or a GI increment of >10 between two consecutive GI readings. Gram-stained smears were prepared from positive bottles, and samples of the broth were subcultured onto blood and chocolate agar media, MacConkey agar, and New York City medium. Terminal subcultures of negative bottles were not performed. Identification of the isolated organisms was performed by standard bacteriological procedures (5). Identification of *Brucella* spp. was based on a typical Gram's stain and colonial morphology; no growth on MacConkey agar; positive oxidase, catalase, and urease tests; negative fermentation of sugars; and positive agglutination with specific antiserum (Welcome Diagnostics, Dartford, England). The isolated *Brucella* strains were sent to the Israel Veterinary Institute at Beth Dagan for further identification and determination of their biotypes.

(ii) Cultures for *Brucella* spp. When the attending physicians suspected brucellosis on the basis of clinical and/or epidemiological considerations, a special written requisition form was added. The blood culture protocol for *Brucella* spp. for the first 7 days was identical to that for routine blood cultures. At the end of the first week, anaerobic bottles were discarded and aerobic bottles were kept for an additional 3 weeks and were monitored by the BACTEC machine on days 14, 21, and 28. In addition, blind subcultures of samples from the blood culture

TABLE 1. Time for detection of *B. melitensis* in routine and special cultures for *Brucella* spp. by the BACTEC machine or by blind subculture

Culture	No. of:										
	Cultures	Positive cultures	Patients	Cultures positive by:							
				BACTEC GI on day[a]:				Blind subculture on day:			
				1	4	5	7	7	14	21	28
Routine	36,890	42	27	2	6	15	19	ND[b]	ND	ND	ND
For *Brucella* spp.	373	27	21	0	2	7	12	0	5	1	0

[a] GI, growth index criteria.
[b] ND, not done.

bottles were performed on days 7, 14, 21, and 28, disregarding the GI readings.

RESULTS

During the 24-month study period, 36,890 blood culture sets, which included a 6NR bottle and for which no special requests for *Brucella* sp. cultures were formulated, were processed. Forty-two of them (0.1%), obtained from 27 different patients, grew *B. melitensis*. Cumulative detection of the organism by the BACTEC machine was in 2 (4.8%) cultures by day 2, 8 (19.0%) cultures by day 4, 23 (54.8%) cultures by day 5, and 42 (100%) cultures by day 7 (Table 1).

The organism was recovered from a significantly higher ($P < 0.001$) proportion of cultures for *Brucella* spp. than from routine cultures. Culture for *Brucella* spp. was requested for 373 blood cultures, and 27 of them (7.2%), drawn from 21 different patients, were positive for this organism. The cumulative detection rate was 0 of 27 positive cultures by day 2, 3 (11.1%) by day 4, 7 (25.9%) by day 5, and 21 (77.8%) by day 7. For the remaining six blood cultures, the organism was recovered from blind subcultures of samples from bottles which did not meet the GI criteria for positivity after 7 days of incubation. In none of these six cases were brucellae detected in subcultures performed at the end of the first week. In five of these cases the organism was recovered in blind subcultures performed on day 14, and in one of these cases the organism was detected in a blind subculture on day 21.

GIs rose slowly, and by the time of detection by the BACTEC machine the highest recorded GI was 78. In 5 of the 69 positive cultures, bacterial growth was detected only by an increase of ≥10 GI units between two consecutive readings. Despite the low GI values that were recorded, Gram's stain of the broth frequently disclosed the presence of abundant microorganisms and even of microcolonies.

All isolates were found to belong to biotype 1, which is the prevalent *B. melitensis* biotype in Israel.

DISCUSSION

As the result of effective infection control measures implemented by health authorities, brucellosis has become a rare disease in North America and parts of western Europe. Because of that, published experiences with modern blood culture techniques, and particularly with the BACTEC system, for the detection of *Brucella* organisms are limited to a few reports of small outbreaks of the disease among travelers to countries where brucellosis is endemic (3, 9).

In 1984, Arnow et al. (3) reported that the radiometric BACTEC 460 system yielded *B. melitensis* in 15 of 19 blood culture sets obtained from a group of six patients involved in an outbreak of disease among travelers to Spain. A positive radiometric reading was detected by the blood culture instrument between 4 and 8 days after inoculation for all positive bottles.

In 1991, Zimmerman et al. (9) reported a case of brucellosis in a visitor to Mexico, in which *B. abortus* bacteremia remained undetected by the nonradiometric BACTEC NR730 instrument for 7 to 20 days. In the two initial blood culture sets, the organism was recovered only from blind subcultures. Seeded culture studies with the original isolate indicated that a large inoculum of *B. abortus* (500 CFU/ml) was detected by the BACTEC instrument within 2 days, whereas 5.5 to 7.5 days were required to detect a small inoculum (5 CFU/ml). This simulated blood culture experiment suggested that the BACTEC NR system may lack the capability of detecting low numbers of circulating *Brucella* organisms within the time frame of a routine blood culture schedule. The diagnosis of brucellosis will thus be missed in many patients whom the disease is not suspected because, by current laboratory practices, blood culture bottles are not held for more than 1 week and terminal subcultures of negative bottles are not usually performed.

Although the results obtained with simulated *B. abortus* blood cultures imply that the sensitivity of the BACTEC system depends on the concentration of circulating bacteremia, I am not aware of any clinical study on the density of bacteria in patients with brucellosis. Kolman et al. (6) have compared the performances of a lysis concentration method, which allows quantitation of viable organisms in the original blood sample, and the radiometric BACTEC system for the isolation of brucellae. The organism recovery rates of both systems were similar, but the lysis technique had a much shorter detection time (3.5 days [range, 2 to 4 days], versus 14 days [range, 7 to 30 days] for the BACTEC system). Recently, Navas et al. (7) have compared the Isolator lysis-centrifugation system and the BACTEC NR system for the detection of *Brucella* species. The study showed that the sensitivities of both systems were comparable (six of seven patients who were positive by the Isolator system were also positive by the BACTEC system); the detection times, however, differed significantly. The Isolator system had a mean detection time of 3.1 days (range, 2 to 5 days), whereas the BACTEC system had a mean detection time of 20.6 days (range, 17 to 29 days). Unfortunately, in neither study were quantitative blood culture results reported, and therefore, at this time, the results of the experiment of Zimmerman et al. (9) can be confirmed or refuted only on an empirical basis.

The purpose of the present study was to determine the proportion of blood cultures positive for *B. melitensis* that would have been missed by the BACTEC NR660 system after a routine 7-day monitoring period in an area endemic for the organism. To give a full answer to this question, all blood

cultures obtained from febrile patients should have been monitored with periodic blind subcultures for 30 days, as recommended previously (2, 5). Such a study design would have been totally unfeasible because of the large number of blood cultures processed by the Clinical Microbiology Laboratory, the lack of sufficient incubation facilities, the elevated costs, and the unavoidably high contamination rate resulting from the prolonged incubation.

I decided to limit the present study to a well-defined subset of blood cultures consisting of those drawn from patients in whom the diagnosis of brucellosis was suspected on clinical and epidemiological grounds, assuming that the proportion of cultures in which *B. melitensis* would be detected would be particularly high for this population, allowing inclusion of a relatively small number of blood culture sets. Although the fraction of blood cultures positive for *B. melitensis* was significantly higher when samples were drawn from patients with suspected brucellosis, the results of the present study show that even in areas where brucellosis is endemic and where increased awareness of the disease should exist, the vast majority of positive cultures are obtained from patients for whom a diagnosis of brucellosis is apparently not considered. This paradox is partially explained by the fact that the severity of brucellosis disease in humans is extremely variable and the clinical manifestations of brucellosis may be nonspecific and may mimic those of other infectious and noninfectious conditions. The practical implication of this observation is that to optimize the diagnosis of this important disease, any future blood culture system to be used in areas endemic for *B. melitensis* will have to be able to detect the organism within the regular blood culture period.

The results of the present study also show the risks for the laboratory personnel posed by testing biological specimens containing *B. melitensis* (8). Because *Brucella* species are easily transmitted by the aerosol route, they are considered biosafety 3 organisms, and as such, all bacteriological work involving specimens possibly contaminated by the organism should be performed in a safety cabinet. In the course of the study, however, one of the laboratory technicians acquired the disease while processing a blood culture obtained from a patient in whom the diagnosis of brucellosis was not suspected.

Brucellosis may be a serious health hazard to populations living in areas where the disease is endemic. The results of the present study show that, in the majority of cases, the BACTEC NR660 system allows recovery of the organism within the routine 1-week blood culture incubation period and may help to establish an early diagnosis of the disease and the early administration of specific antimicrobial therapy. These results also demonstrate that, when using the BACTEC NR660 blood culture system, failure to detect circulating *B. melitensis* after 7 days will usually predict a negative blood culture result after 4 weeks. To maximize the detection of brucellosis, however, incubation of bottles and performance of blind subcultures for 3 consecutive weeks are still recommended whenever a diagnosis of brucellosis is suspected.

REFERENCES

1. **Abramson, O., Z. Rosenvasser, C. Block, and R. Dagan.** 1991. Detection and treatment of brucellosis by screening a population at risk. Pediatr. Infect. Dis. J. **10:**434–438.
2. **Alton, G. G., and L. M. Jones.** 1967. Bacteriological methods, p. 17. *In* Laboratory techniques in brucellosis. World Health Organization, Geneva.
3. **Arnow, P. M., M. Smaron, and V. Ormiste.** 1984. Brucellosis in a group of travellers to Spain. JAMA **251:**505–507.
4. **Gotuzzo, E., C. Carrillo, J. Guerra, and L. Llosa.** 1986. An evaluation of diagnostic methods for brucellosis: the value of bone marrow culture. J. Infect. Dis. **153:**122–125.
5. **Hausler, W. J., Jr., N. P. Moyer, and L. A. Holcomb.** 1985. Brucella, p. 382–386. *In* E. H. Lennette, A. Ballows, W. J. Hausler, Jr., and E. H. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
6. **Kolman, S., M. C. Maayan, G. Gotesman, L. A. Rozenszajn, B. Wolach, and R. Lang.** 1991. Comparison of the BACTEC and lysis concentration methods for recovery of *Brucella* species from clinical specimens. Eur. J. Clin. Microbiol. Infect. Dis. **10:**647–648.
7. **Navas, E., A. Guerrero, J. Cobo, and E. Loza.** 1993. Faster isolation of *Brucella* spp. from blood by Isolator compared with BACTEC NR. Diagn. Microbiol. Infect. Dis. **16:**79–81.
8. **Staszkiewicz, J., C. M. Lewis, J. Colvile, M. Zervos, and J. Band.** 1991. Outbreak of *Brucella melitensis* among microbiology laboratory workers in a community hospital. J. Clin. Microbiol. **29:**287–290.
9. **Zimmerman, S. J., S. Gillikin, N. Sofat, W. R. Bartholomew, and D. Amsterdam.** 1990. Case report and seeded blood culture study of *Brucella* bacteremia. J. Clin. Microbiol. **28:**2139–2141.