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Diagnosis of Brucellosis by Using Blood Cultures

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The performances of three blood culture systems, Hémoline performance diphasic medium (bioMérieux, Marcy l'Etoile, France), Bactec Plus Aerobic/F* (Becton Dickinson, Paramus, N.J.), and Vital Aer (bioMérieux), were compared for the diagnosis of 17 cases of brucellosis. By using a 5-day incubation protocol, positive results were 52.9, 82.4, and 11.8%, respectively. When the protocol was extended to 7 days, the results were 76.5, 94.1, and 47.1%, respectively. Bactec was the fastest system ($P < 0.05$).

Although typical brucellosis is easily recognized in areas where it is endemic, there are other, more difficult cases which may go unnoticed, especially in those places where incidence of this infection is generally very low. A definitive diagnosis of this infection is based on the culture of *Brucella* strains from different samples, mainly blood. A positive result of the cultures depends on varying factors, including the species of *Brucella*, how advanced the disease is, and whether there has been any previous treatment with antibiotics. Spain, where the predominant species by far (98%) is *Brucella melitensis* (13), is one of those areas of endemicity for this disease together with all the Mediterranean area, the Arabian Peninsula, Mexico, Central America, and South America. In the United States, where the disease is far less frequent, changes in the predominant species have been observed throughout the last three decades. Thus, in the 1970s the highest number of isolates corresponded to *Brucella suis*, in the 1980s it was *Brucella abortus*, and now the most frequently isolated species is *B. melitensis* (3).

Traditionally, *Brucella* species have been cultured in Castañeda medium (14). Results have been satisfactory although the necessary incubation times are very long (6). The more recent use of other diphasic enriched media (Hémoline performance, [bioMérieux, Marcy l'Etoile, France], Septi-Chek [Becton Dickinson, Paramus, N.J.], etc.) has contributed to an improvement in the results (4). 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, 10). However, it has not been shown that these systems are successful in the case of *Brucella* with the same periods of incubation, since available data are insufficient. In this report, we would like to contribute our own experience in the isolation of *Brucella* species from blood, by two different methods of blood culture, a manual diphasic bottle and two automatic fluorescent systems.

During 1995 and 1996, blood culture samples were taken from all those patients who came to the emergency department of the "Virgen de la Arrixaca" University Hospital, in Murcia, Spain, with suspected brucellosis which was confirmed by means of a serodiagnosis of brucellosis by the rose bengal antigen card test (Brucelloslide test; bioMérieux). All the patients were adults, were in the acute phase of the disease, and had not been treated with antibiotics, except case 12, who had taken a dose of tetracycline 4 h before. Only one blood culture was processed per patient. Thirty milliliters of blood from each one was taken and divided equally among three bottles, a Hémoline performance diphasic medium (bioMérieux), a Bactec Plus aerobic/F* bottle (Becton Dickinson), and a Vital aerobic bottle (bioMérieux). The Bactec Plus and the Vital bottles were incubated in their respective automatic systems (Bactec 9120 and Vital systems), while an incubator at 37°C was used for the Hémoline bottle. The three bottles were maintained in this way for 21 days and then subcultured, unless any sign of positiveness had appeared beforehand.

Of the 17 cases of brucellosis under study, Hémoline recovered 17 strains while the two automatic systems each detected 16. These two false-negative results occurred with different patients (Table 1). The subcultures from these bottles carried out after 21 days of incubation were also negative. If the incubation protocol had been only 5 days, Vital would have detected 11.8%, Bactec would have detected 82.4%, and Hémoline would have detected 52.9%. When the incubation protocol was increased to 7 days, the figures were 47.1, 94.1, and 76.5%, respectively. The results of the median, mean, and interval for the three systems are shown in Table 1. The earliest reading was that obtained by the Bactec system after 59 h, while it is interesting to note that in three cases Hémoline was positive after only 72 h. The fastest reading from Vital was 67 h, and this was the only case in which it improved on the time obtained by Bactec (93 h).

Brucellosis is a rare disease in most developed countries, to such an extent that confusion has sometimes arisen over its identification, most probably due to the unexpectedness of the results of its isolation (1, 12). In certain countries like Spain, however, the disease is quite common, although its incidence has decreased quite a lot over the last few years (5).

Several factors affect the growth and detection of *Brucella* species in blood cultures. Gamazo et al. (7) have suggested that the low level of CO_2 released is the most important limiting factor for the detection of *Brucella* species in any given medium. The sodium polyanethol sulfonate, used as an anticoagulant in many blood culture systems, exerts a harmful effect on the outer membrane of the *Brucella* species, making it permeable to hydrophobic substances and thus hindering growth (9). On the other hand, the inoculum is a factor which is inversely proportional to the time of detection (15, 17). These determining factors and others make the isolation and detection of *Brucella* species in culture medium a long and difficult process.

When using the Hémoline bottle, we obtained 100% recovery, but with a maximum time of 216 h. A further advantage of the diphasic bottle is safety, since very little manipulation is needed. By using the Bactec NR 660 system, Yagupsky (16)

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TABLE 1. Results for the 17 cases of brucellosis

Case no.	Time (h) for result with test:		
	Hémoline	Bactec	Vital
1	144		144
2	120	101	160
3	120	93	67
4	72	61	165
5	96	84	97
6	120	90	192
7	96	86	166
8	120	101	174
9	216	123	201
10	72	59	122
11	144	124	193
12	192	117	196
13	168	116	179
14	192	65	221
15	168	94	
16	72	59	173
17	216	105	145
No. of isolations	17	16	16
Median (h)	120	93	166
Mean (h)	136.94	92.37	162.18
No. of isolations (%) in 5 days	9 (52.9)	14 (82.4)	2 (11.8)
No. of isolations (%) in 7 days	13 (76.5)	16 (94.1)	8 (47.1)

isolated 21 of 27 *B. melitensis* strains by blind subcultures in under 7 days. The remaining six required up to 3 weeks. Recently, Nizar et al. (11) improved on these results by using the more modern fluorimetric Bactec system, without any final subculture being necessary. The system detected all 19 strains of *Brucella* in their study in an interval of 4 to 12 days (average, 8 days). Our experience with the same Bactec system was even better (2.5 to 5 days; average, 3.85 days). We do not know to what the differences are due, but we think that one reason might be that their patients, whom they do not define, either were not in the acute phase of disease or had been treated with antibiotics. By using the Vital system, we obtained the same percentage of recovery as the Bactec system (94%), but with clearly longer detection times. Very different results have been published about Bac-Alert, another automatic system similar to the ones used by us. Thus, Solomon and Jackson (15) described a case of early detection (2.8 days) with this system, while Casas et al. (2) needed 10 to 20 days to recover all of the seven strains from their study, after a blind subculture.

In summary, the Bactec system detected the brucellas in all cases, except one, sooner than the Vital system ($P < 0.05$). This latter system was sometimes slower than even the manual diphasic system. The incubation protocol for quick processing (5 days) recommended for conventional bacteria is on the border of detection time of the fastest system (Bactec) tested by us. By using a 7-day incubation protocol, Bactec detected 94.1% of the strains, while Vital detected only 47.1%. This figure is even lower than that of the manual Hémoline system (76.5%). Thus, it seems reasonable to extend incubation time when brucellosis is suspected, at least when the Vital or Hémoline system is used. Our data from Bactec, on the other hand, indicate that 7 days is enough, although it would be interesting to investigate what would happen with patients who were being treated with antibiotics. Considering our experience and the data available, we believe that each automatic system should be studied separately to establish an optimum incubation period for this microorganism and thus avoid overall rules which may be validated for other bacteria.

Finally, physicians should bear in mind these difficulties in the isolation of *Brucella* species when requesting blood cultures. It would be of great help if they were to specify the suspected etiologic agent, since this may be decisive in many cases to achieve the microbiological diagnosis.

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ADDENDUM

During the period of revision and correction of the manuscript, we had two new cases of brucellosis with the following results: for case 18, 144, 76.18, and 131.17 h for the Hémoline, Bactec, and Vital tests, respectively, and for case 19, 312, 114.73, and 180.32 h for the Hémoline, Bactec, and Vital tests, respectively.

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