Introduction

Brucellosis is a zoonosis of worldwide importance\(^1,2\) with well described manifestations and complications.\(^3,4\) The diagnosis of brucellosis requires the isolation of organisms from blood or body tissues, or the combination of suggestive clinical presentation and positive serology. However, in endemic areas culture is required for a definitive diagnosis because the symptoms and signs of brucellosis are non-specific, and the interpretation of agglutinating antibody titre can be confounded by persistently elevated titre in persons without active disease who have been repeatedly exposed to infected animals.\(^5\)

Brucellosis is a systemic infection in which the bacteria initially localize in the regional lymph nodes, then disseminate haematogenously to the organs of the reticuloendothelial system to multiply within phagocytic cells.\(^6\) The release of bacterial endotoxin from phagocytic cells produces the constitutional symptoms and signs of the disease, and may explain in part why not all febrile brucellosis patients are bacteraemic and why not all bacteraemic patients are febrile.\(^7\) Brucellosis is an intracellular infection so bacteraemia, though not present in all infected patients, provides the best opportunity to study the efficacy of anti-\textit{Brucella} chemotherapy in humans.\(^8\) We report a 12-year experience with 160 cases of \textit{Brucella} bacteraemia diagnosed at King Fahad National Guard Hospital, Riyadh, Saudi Arabia.

Patients and methods

The King Fahad National Guard Hospital provides secondary and tertiary medical care to Saudi National Guard soldiers and their extended families. Many of these people, who retain traditional lifestyles, live in close association with \textit{Brucella}-infected sheep, goats, and camels, and consume unpasteurized milk or cheese from these animals.\(^9,10\) Patients with a discharge diagnosis of brucellosis from 1983 to 1995 were identified using the hospital medical record database, and their charts were reviewed. All patients with \textit{Brucella} bacteraemia were included in our study.

Brucella Bacteraemia: Clinical and Laboratory Observations in 160 Patients

Ziad Memish\(^*1,2\), Manuel W. Mah\(^1,2\), Suliman Al Mahmoud\(^1\), Mohammad Al Shaalan\(^3\) and M. Yousuf Khan\(^1,2\)

Department of 1Medicine, 2Infection Prevention & Control, and 3Pediatrics, King Fahad National Guard Hospital, Riyadh, Kingdom of Saudi Arabia

Objectives: To describe the clinical, serological, and prognostic features of bacteraemic brucellosis in an endemic region.

Methods: Retrospective case series of 160 patients admitted from 1983 to 1995 to a hospital providing secondary and tertiary level medical care in Saudi Arabia. All patients had positive blood cultures for \textit{Brucella} species, predominantly \textit{Brucella melitensis}.

Results: Bacteraemia was documented in 38% of 545 cases of brucellosis admitted to our institution during the study period. The main clinical syndromes were febrile illness alone (44%) or fever with arthritis (42%). Of 68 isolates that were speciated, 93% were \textit{Brucella melitensis}. Initial agglutinating antibody titre was \(\geq 1:320\) in 96% of the patients. Antimicrobial resistance of \textit{B.melitensis} isolates was: co-trimoxazole, 29%; rifampicin, 3.5%; streptomycin, 0.6%; and tetracycline, 0.6%. No increase in resistance was noted over the 13-year study period. Commonly used antimicrobial regimens consisted of streptomycin plus tetracycline or rifampicin plus doxycycline given for 6 weeks. Seven patients (5%) had relapse of their symptoms after antimicrobial therapy. Three of these had infective endocarditis with repeated bacteraemia. These patients required aortic valve replacement and recovered after surgery. The remaining four patients responded to a second course of therapy.

Conclusions: \textit{Brucella} bacteraemia is an acute febrile disease often associated with rheumatologic complaints. Most patients have an agglutinating antibody titre \(\geq 1:320\) and respond well to standard chemotherapy regimens with low mortality.

\(^*\)Correspondence to: Dr. Ziad Memish

Accepted for publication 9 August 1999.

\(0163-4453/00/01059 + 05 \$ 35.00/0\)

© 2000 The British Infection Society
the study. Information on demographics, clinical presentation, serology and blood culture results, antimicrobial susceptibility, treatment, complications, and clinical course were extracted.

A variety of microbiological methods were used to isolate Brucella during the 12-year study period. From 1983 to 1985, blood and other body fluids were cultured initially in tryptic soy broth and thiol broth bottles with 5% CO₂, and subcultured onto chocolate agar, blood agar, and enriched modified blood agar for anaerobes. In 1986, the laboratory began to use the BACTEC 660 system (Becton-Dickinson, Towson, Maryland, U.S.A.) for initial blood cultures with subculture onto enriched media and speciation by biochemical methods: CO₂ requirement, urease activity, and growth on basic fuchsin and thionin dyes. In 1995, the BACTEC 660 system was replaced by the BACTEC 9240 system for initial blood cultures with subculturing onto chocolate blood agar at 37°C in CO₂.

Antimicrobial susceptibility testing was performed by broth dilution; antimicrobial powders were weighed and diluted at 1280 µg/ml in sterile, deionized water (except for trimethoprim-sulfamethoxazole, which was prepared to contain 1000 µg of trimethoprim and 19,000 µg of sulfamethoxazole per ml). An equal volume of diluted broth culture of the isolate was added to each antimicrobial dilution yielding a final inoculum of 5 × 10⁵ CFU/ml in a final volume of 5 ml. Incubation was at 35 – 37°C. Readings were made each day for 7 days to determine the extent of inhibition. Only minimum inhibitory concentration was determined for each antimicrobial.

Titres of Brucella agglutinating antibodies were measured by a microtitre agglutination procedure using Brucella abortus and Brucella melitensis antigens (stained B. abortus SS14 and B. melitensis SS15 suspensions containing approximately 10¹⁰ organisms per ml; Wellcome Diagnostics, Dartford, England); all sera were routinely diluted from 1:80 to 1:20,480 to overcome prozone phenomenon. Each batch of tests included a positive 1:1,280 control and a negative saline control. A definite agglutination of the suspension was read as a positive reaction. If prozone phenomenon was encountered, the higher dilution agglutination was recorded.

Various treatment combinations of the five anti-Brucella antimicrobials (streptomycin, tetracycline, doxycycline, co-trimoxazole, rifampicin) were used depending on the patient’s age, pregnancy status, and whether they had central nervous system involvement. Tetracycline and doxycycline were not administered to children less than 10 years of age or to pregnant patients. Rifampicin and co-trimoxazole were the only antimicrobials prescribed to pregnant patients. Streptomycin was avoided in patients over the age of 60 years. Patients with central nervous system involvement received three to four antimicrobials. Dosage was according to standard recommendations for paediatric and adult patients. Mean follow-up period was 6 months. Long-term follow-up was not possible because of the nomadic nature of the patient population. Relapse was defined as initial improvement with reappearance of symptoms and signs during the treatment period or within 2 months after discontinuation of treatment. Cure was defined as no recurrence of symptoms and signs during the 6 month follow-up period.

The statistical significance of sex proportions was assessed with the z normal statistic. The proportions of women aborting in different trimesters were compared using the Fisher exact test. The time trend in co-trimoxazole resistance was assessed with the chi-square test for linear trend in proportions.

Results

From 1983 to 1995, there were 545 patients diagnosed with brucellosis at our institution, of which 420 had blood cultures drawn. One hundred and sixty (38%) of these patients (119 adults, 41 children ≤ age 12 years) had Brucella bacteraemia and were included in the study. Non-bacteraemic cases were diagnosed on the basis of symptoms suggestive of brucellosis and a serological titre of 1:320 or greater. One hundred and thirty-seven patients (86%) with bacteraemia were hospitalized from 1984 to 1988, while 23 (14%) were hospitalized from 1989 to 1995. Analysis of the monthly distribution of hospitalizations revealed that 135 patients (84%) were admitted during the months of March to October, while 25 (16%) were admitted during November to February. The highest number of admissions occurred in March and July, and the lowest number occurred in January.

Among 119 adults with Brucella bacteraemia, 56% were male, and among 41 children (age ≤12 years) with bacteraemia, 56% were male; the excess proportions of males over females were not statistically significant (P>0.05). The mean age of all patients was 30 years and 71% of patients were ≤40 years age.

The presenting symptoms, signs, and clinical syndromes of 160 bacteraemic patients are shown (Table I). Among 153 patients in whom duration of symptoms prior to hospitalization was obtained, 114 (75%) had duration less than or equal to 2 weeks, 29 (19%) had duration greater than 2 weeks but less than or equal to 6 weeks, and 10 (6%) had duration greater than 6 weeks. The mean duration in those with symptoms greater than 6 weeks was 86 days. Arthritis was present in 68 (42%) patients and nine of these patients had osteomyelitis diagnosed radiologically (Table II). Twenty-two of the 160 bacteraemic patients were pregnant, and nine (41%) had
spontaneous abortions (One of three women in the first trimester, seven of thirteen women in the second trimester, and one of six women in the third trimester aborted; comparison of the proportions of women aborting in the three trimesters was not statistically significant ($P > 0.05$ for all comparisons). Only 68 of the 160 Brucella isolates were speciated because some isolates died, some specimens were discarded, or laboratory reagents were not available. Of the 68 isolates, 63 (93%) were $B. melitensis$ and five (7%) were $B. abortus$. Antimicrobial susceptibility testing was carried out on 143 (90%) isolates. None of the $B. abortus$ isolates were resistant to anti-Brucella antimicrobial agents, but the number of isolates was small. The number of $B. melitensis$ isolates with antimicrobial resistance was as follows: co-trimoxazole, 40 (29%); rifampicin, five (3.5%); streptomycin, one (0.6%); and tetracycline, one (0.6%). The five isolates with rifampicin resistance all occurred in different years. The 40 isolates with co-trimoxazole resistance (Fig. 1) did not show a statistically significant trend of increasing resistance with time ($P = 0.2$).

Of 160 bacteraemic patients, 155 had Brucella serology performed on admission (Fig. 2). The initial titre was $\geq 1:320$ in 149 patients (96%), 1:160 in one patient, and $\leq 1:80$ in five patients. Of the six patients who had initial titre $\leq 1:160$, four had four-fold or higher titres on subsequent testing and two were not re-tested.

Treatment regimens used in the 160 patients consisted of combinations of two or three agents: streptomycin plus tetracycline (36%), doxycycline plus rifampicin (17%), streptomycin plus tetracycline plus rifampicin (17%),
rifampicin plus co-trimoxazole (14%), streptomycin plus rifampicin plus co-trimoxazole (9%), and other combinations (7%). Streptomycin was given for 10–15 days, and other antimicrobial agents were used for 6 weeks.

Of 160 patients, 22 failed to return for a follow-up visit after improvement of symptoms during their hospitalization. The remaining 138 patients returned for follow-up with a mean duration of follow-up of 6 months. Of these 138 patients, 131 were cured of their symptoms with a single treatment course during the 6 month follow-up period. Seven patients had a relapse of their symptoms during the treatment period or within 2 months after discontinuation of treatment. Three of these relapses were due to Brucella endocarditis, and these three patients recovered after chemotherapy and aortic valve replacement. The four remaining patients responded to a second course of therapy.

**Discussion**

There are few case series of *Brucella* bacteraemia in the literature.7,16 A Medline search for the years 1975 to 1997 using the search terms ‘brucellosis’ and ‘bacteraemia’ did not yield any other series, although many series of brucellosis cases mention some patients with positive blood cultures in passing. The yield of blood cultures in brucellosis ranges from 15 to 70%,17, and some specific reported isolation rates are 691, 35,18, and 29%.11 In our institution, bacteraemia was detected in 38% of all hospitalized brucellosis cases during the study period.

The annual incidence of *Brucella* bacteraemias presenting to our hospital has declined with time, with a much smaller proportion of cases occurring after 1988. This declining incidence cannot be attributed to changing bacteriologic methods because the BACTEC 660 system was in use at our hospital from 1986 to 1995. More likely, the declining incidence can be attributed to increased awareness of the disease among physicians, improved laboratory diagnosis, and treatment of a higher proportion of cases by primary care physicians so that these patients do not present to hospital.19 In addition, public health measures such as public education, milk pasteurization, and livestock immunization may also have contributed.

The incidence of bacteraemic brucellosis is highest in spring and summer, and lowest in the winter months. This seasonal variation has been observed in other brucellosis series18–20 and has been(120,831),(924,840)
confirmed cases, a titer of 1:320 has good positive predictive value. Six patients with bacteremia had titres ≤1:160 and five patients had titres ≤1:80; this was due to early presentation of the disease, because in four patients who were subsequently retested, all had a four-fold or higher rise in titre. Hence, neither a low initial agglutination titre nor absence of fever necessary rules out Brucella bacteremia. An earlier study in our patient population did not reveal any significant difference in titre between bacteremic and non-bacteremic patients.

In conclusion, Brucella bacteremia in our setting is an acute febrile disease often associated with rheumatologic complaints. Most patients with bacteremia can be diagnosed by an agglutination titre ≥1:320 and respond well to standard chemotherapy regimens with low mortality.

References