Identification of Dye-Sensitive Strains of *Brucella melitensis*

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Strains of *Brucella melitensis* biovars 1, 2, and 3 isolated in India, Italy, Kuwait, Saudi Arabia, the Federal Republic of Germany, and Zimbabwe were inhibited by thionin (20 μg/ml) but not by basic fuchsin (20 μg/ml) or safranin O (200 μg/ml). In other respects the strains were typical of their species and biovars. The existence of dye-sensitive strains of *B. melitensis* suggests that the present system of classification requires modification.

Although molecular genetic studies have indicated that the genus *Brucella* comprises a single species (1, 13, 14, 16), it is currently classified into six nomen species which correlate fairly closely with their preferred natural hosts (8, 15). The three most important species in terms of worldwide prevalence and production of morbidty in human beings and domestic animals, *Brucella melitensis*, *B. abortus*, and *B. suis*, are subdivided into biovars on the basis of cultural, biochemical, and serological differences in the case of the last two but solely on the basis of the serological specificity of the surface antigens in the case of *B. melitensis* (8). In effect, these three recognized subtypes of *B. melitensis* are serovars rather than biovars. That this system of differentiation does not adequately reflect the differences which exist between many strains of *B. melitensis* has been recognized for some years (8). Thus, Arnaud-Bosq and colleagues showed that *B. melitensis* could be subdivided into five major groups on the basis of oxidative metabolism tests with six selected substrates (2, 3). Representatives of the three currently recognized biovars (serovars) were distributed between these groups. Gargani and Tolari reported that subtypes within *B. melitensis* biovar 2 could be identified by differences in phage sensitivity (12). They also identified strains of this biovar which showed unusual patterns of sensitivity to the dyes basic fuchsin and thionin. More recently, Banai and others have reported the existence of a subtype within *B. melitensis* biovar 1 distinguished by its sensitivity to basic fuchsin and thionin (4).

These reports prompted an examination of *B. melitensis* cultures held in the collection of the Food and Agricultural Organization/World Health Organization Collaborating Centre for Reference and Research on Brucellosis at the Central Veterinary Laboratory, Weybridge, United Kingdom, for unusual patterns of dye sensitivity.

Examination of typing data on approximately 500 strains of *B. melitensis* received between 1980 and 1986 from a large number of European, Asian, African, and South and Central American countries in which infection with this organism is known to be prevalent identified 29 strains with anomalous dye sensitivity. These strains, isolated from human and animal sources in six countries in Europe, Asia, and Africa (Table 1), were selected for reexamination.

Culture typing was performed by conventional procedures for determination of morphology; cultural characteristics; CO₂ requirement; H₂S and urease production; sensitivity to inhibition by dyes; agglutination with antisera specific for A, M, and R antigens; and susceptibility to antibiotics and lysis by brucella phages (7). Oxidative metabolism tests (8) were done on selected strains to confirm the species identification by phage typing.

All strains were typical of field isolates of *B. melitensis* in morphology, colonial appearance, and growth characteristics. None was dependent on CO₂ for growth, and none produced more than traces of H₂S. Their urease activities showed the range of variation typical of *B. melitensis* (9). The strains conformed to either phage typing pattern B or C in the classification of Corbel (6) (pattern C is that typical of most smooth *B. melitensis* strains and is distinguished by lysis by phages BK₅ and Iz only, at routine test dilution; pattern B is similar, but lysis also occurs with phage Wb at routine test dilution). The oxidative metabolism pattern was typical of *B. melitensis*. All strains behaved as smooth cultures in tests for dissociation and were not agglutinated by antisera to R antigen. In agglutination tests with antisera monospecific for the A and M epitopes of *Brucella* smooth lipopolysaccharide antigens, the strains gave patterns typical of *B. melitensis* biovar 1, 2, or 3, with the majority identified as biovar 1 (Table 1). In dye sensitivity tests, all strains were completely resistant to basic fuchsin at 20 μg/ml but were inhibited by thionin at the same concentration. All strains were resistant to safranin O at 100 and 200 μg/ml and were also resistant to i-erythritol at 1 and 2 mg/ml.

Their antibiotic susceptibility patterns were typical of field isolates of *B. melitensis*, and the strains were resistant to standard concentrations of benzylpenicillin but susceptible to streptomycin and other aminoglycosides, including amikacin, gentamicin, sisomycin, and tobramycin. With the exception of the strains of Kuwaiti origin, which were unusual in being in large proportion phage type B, the isolates all showed similar ranges of properties, irrespective of geographic origin.

The sensitivity to thionin was independent of whether the strains were grown in CO₂ or in air, and there was a clear-cut difference from typical *B. melitensis* strains, including the reference strains, all of which produced vigorous growth on both thionin and basic fuchsin. No evidence of sensitivity to basic fuchsin was observed and these strains were clearly distinct in properties from those described by Banai et al. (4).

Although dye sensitivity represents but a single phenotypic characteristic, it forms the basis for the differentiation of some *B. abortus* biovars (7, 8, 15). The occurrence of

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The present observations confirm and extend those of others which have indicated that differences between *B. melitensis* strains are not confined to agglutination pattern alone. This suggests that the present criteria used for determination of biovars should be reexamined.

**REFERENCES**


