

MALDI-TOF MASS SPECTROMETRY DOES NOT DIFFERENTIATE BETWEEN BRUCELLA SPECIES, SUGGESTING THAT THEY MAY REPRESENT A SINGLE SPECIES

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At present, eight species of *Brucella* has been described based on traditional, phenotypically based criteria (host preferences, metabolism, culture and antigenic features), and six of them have been involved in human infections. However, DNA relatedness and multilocus enzyme electrophoresis indicate a high homology between species, suggesting all brucellae might really represent a single species. The aim of the present study was to analyze protein expression of *Brucella* species using MALDI-TOF mass spectrometry. We created MALDI-TOF spectra using type strains of the six species involved in human infections (3 *B. melitensis*, 5 *B. abortus*, 1 *B. suis*, 1 *B. canis*, 1 *B. ceti* and 1 *B. pinnipedialis*). The visual inspection of the mass spectra from whole-cell extract revealed a high similarity among them, with specific peaks common for all the strains. Although some differences can be observed among all the strains, only some strains have discriminating peaks at species levels. According these data, there is a peak profile characteristic of *Brucella* to the genus level, but there is not to the species level. A cluster analysis has provided a dendrogram which shows a close proximity between *B. ceti* and *B. pinnipedialis*, and between *B. suis* and *B. canis*, and more proximity between these four species than between any of them and *B. melitensis* and *B. abortus*. Otherwise, *B. melitensis* and *B. abortus* biotypes are extremely close and intermingled. When we used generated database for *Brucella* species with clinical isolates, both from agar plate cultures and directly from blood cultures, the results obtained showed that these profiles allow a reliable identification of brucellae to the genus level, but not to the species level. Our data are in accordance with taxonomic reports suggesting, on the base of molecular methods, that the 8 currently accepted species represent a single species.