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Biological and molecular characterization of *Brucella canis* in two dog breeding farms of Argentina

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Brucella canis is a worldwide identified bacteria, responsible of infertility and abortion in dogs, with a relative relevance on public health, since occasionally it causes brucellosis in humans. *B. canis* has high genetic diversity and has been mainly confused with *B. suis*. To verify the presence of *B. canis* in two aborted canine foetus from two dog breeding farms geographically distant of Argentina, serum samples, amniotic fluid, stomach content, and lung tissue were analyzed. Serum samples were tested by agglutination test. Samples from fluids and tissues were analyzed by Stamp stain and cultured in commercial *Brucella* agar with 8% bovine blood and Mac Conkey agar incubated under aerobic atmosphere at 37°C during four days. Bacteria from selected colonies were analyzed by morphology, Stamp and Gram stains, catalase, oxidase, urease, citrate utilisation and motility tests. DNA from the growing colonies was purified by conventional phenol-chloroform methods and amplified by combinatorial PCR using different set of primers. The DSF-DSR primers were used to amplify variable sequences from *omps2* genes in the different *Brucella* species. The specific primers BrCF-BrCR, BabF-416R, BmF-416R, BsuisF-416R were used to identify *B. canis*, *B. abortus*, *B. melitensis* and *B. suis* respectively. DNA fragments were visualized by staining with ethidium bromide in 1.5% agarose gel using an ultraviolet transilluminator. Agglutinating antibodies to *B. canis* were detected in serum samples. Typical translucent non-haemolytic colonies were observed after 72 h incubation, only in *Brucella* agar with 8% bovine blood. Gram negative non motile cocobacillus were negative to oxidase, and positive to catalase and urease tests. The combinatorial PCR confirmed the identity of both isolates as *B. canis*. A fragment of 858 bp was amplified from *omp2* gene in both strains; the sequence analysis showed 100% of identity with *B. canis* genome by performing a blast search in the GenBank database. Using specific primers to identify *B. canis* (BrCF-BrCR), two fragments of ~2120 bp and one faint fragment of ~887bp were identified by electrophoresis. The former is found in different *Brucella* species and the second is characteristic of most *B. canis* isolates. Using specific primers, amplicons with the expected sizes were observed for *B. abortus*, *B. melitensis* and *B. suis*, but they were unable to amplify DNA from *B. canis*. We concluded that the combinatory PCR allowed us to specifically identify two *B. canis* isolates.

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