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Specificity of PCR and high resolution melting to identify *B. abortus* wild type and strain 19.

Carolina Thompson[1], Ana Russo[2], Diana Martinez[3], Armando Delgado[4], Vilma Canavesio, Paola Amherdt[1], Susana Torioni de Echaide[1]

[1]INTA EEA Rafaela, Santa Fe, Argentina. [2]CEDIVEF, Formosa, Argentina. [3]Facultad de Ciencias Veterinarias-UNNE, Corrientes, Argentina. [4]Facultad de Ciencias Veterinarias-UNL, Santa Fe, Argentina.
cthompson@rafaela.inta.gov.ar

In Argentina cattle brucellosis, almost exclusively caused by *Brucella abortus*, is under control and eradication program. The diagnosis is based on serological tests and the bacteriological isolation to typify the strains is not routinely used because is cumbersome and time consuming. The usefulness of PCR to support the serological tests of official program has not been defined yet. The specificity of PCR and High Resolution Melting (HRM) to discriminate nucleotide changes by the melting temperature (Tm) of the double strand DNA amplified by real time PCR (RTPCR) was evaluated. DNA from *B. abortus*, *B. suis*, *B. melitensis* and *B. canis* isolated from different regions of Argentina, were analyzed. *B. abortus* strain19 (S19) and DNA from blood samples of cattle (n=6) positive to brucellosis by complement fixation test, were also tested. Different set of primers previously described were used to detect *Brucella* spp. (B4-B5), *B. abortus* (416F-IS711) and from the *eri* gene to distinguish wild type (WT) and S19 (*Eri1-Oligo2* and *Oligo1-Oligo2*). DNA sequences amplified from cattle were compared by BLAST to *Brucella* spp. sequences in GenBank database. Sizes and Tm of products amplified by B4-B5 were 223bp and 87,6ºC±0.07°C from all *Brucella* species analyzed; by 416F-IS711 were 498bp and 88,13°C±0.04°C from both *B. abortus* strains, and were 250bp and 85.61ºC from *B. suis. Eri1-Oligo2* generated fragments of 1000 bp and 90.65ºC from *B. abortus* WT, *B. canis and B. melitensis,* or 297bp and 91.25ºC from S19. *B. suis* showed both faint bands and 91.40ºC. Similarly, *Oligo1-Oligo2* produced fragments of 1026bp and 90.15ºC from *B. abortus* WT, *B. melitensis* and *B. canis* or 365bp and 91.49ºC from S19. *B. suis* also showed both faint fragments and 91.44ºC Tm. Since similar fragments from *eri* gene could be amplified from other *Brucella* species, the specificity should be confirmed by amplifying the 498bp specific for *B. abortus*. The 416F-IS711 primers were less sensitive than those from *eri* gene. The sequencing analysis of *eri* fragments was confirmed as WT or S19 in cattle. Blood samples are not the best target to look for *Brucella* spp. and serology remains as the most rational tool for brucellosis control, therefore PCR and RTPCR should help to define the status of herds with *B. abortus* infection supported by epidemiological criteria. Primers designed to identify other *Brucella* species would be helpful to improve the diagnosis. HRM was useful to identify *Brucella* spp. and *B. abortus,* but useless to discriminate the WT from S19.

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