Medicina Veterinária

The use of Brucella abortus acetone inactivated as antigen in an Indirect ELISA platform for the diagnosis of bovine brucellosis

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Resumo

The Brucella abortus causes an important zoonotic disease in bovines, besides economic lost. Brucellosis diagnosis is based on serological methods and a recent systematic review and meta-analysis (Andrade et al, 2024) showed that the Indirect Enzyme Linked Immunosorbent Assay (iELISA) has high diagnostic sensitivity (DSe) and scalability. Although, test standardization is poorly elucidated. Therefore, this study aims to validate and evaluate the accuracy of an iELISA using the B. abortus acetone inactivated as antigen, taking to account the interference of vaccine antigens and cross-reactions. The B. abortus strain 544 (ATCC 23448) was cultivated in Tryptic Soy Agar (TSA) for 48h, at 37ºC and 5% CO2. After this, the acetone inactivated antigen was produced as described by Colby et al., 2002. A spectroscopy analysis using 785 nm laser and 10- to 50-fold objectives on the Witec Alpha300 Confocal Raman Microscopy System (Oxford Instruments, United Kingdom) was performed to evaluate the antigen chemical structure and then assessed for its potential in the iELISA. The 263 bovine sera samples used for validation of the test were divided into eight groups: Group 1 – 28 samples from non-vaccinated animals in herds with brucellosis outbreak (RBT and 2ME positive); Group 2 – 30 samples from heifers vaccinated with S19 collected 30 days after vaccination; Group 3 – 28 samples from heifers vaccinated with S19 56 days after vaccination; Group 4 – 30 samples from heifers vaccinated with RB51 collected 28 days post-vaccination; Group 5 – 30 samples from heifers vaccinated with RB51 56days post vaccination; Group 6 – 43 samples from culture-positive in natural infected animals; Group 7 – 32 samples from brucellosis-free herds; Group 8 – 42 samples from animals inoculated with inactivated Yersinia enterocolitica O:9 antigen . It was observed that the wave peak of the spectroscopy was 1378 cm-1. The cut-off in the optical density value was 0.2266 having a DSe of 79.91% (CI 99%: 13.02 - 99.75%) and a DSp of 79.77% (CI 99%: 12.79 - 99.75%), both lower than expected for a diagnosis test as an iELISA. Finally, this antigen did not demonstrate good performance in a diagnostic testing platform, showing low DSe or DSp values, indicating a poor capacity to correctly discriminate healthy animals from infected, recently vaccinated animals or cross-reactivity with antibodies against Y. enterocolitica O:9, possibly related to changes in chemical structure from the inactivation method.

Palavras-Chave: Serological test, Accuracy, Diagnostic sensitivity.
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Link do pitch: https://youtu.be/BDuuEHPqxtw