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Evaluation of the cross-reactive antibodies among *Brucella* strains: validation of serological detection tests of Brucellosis

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Abstract

The present study aimed to investigate the antibody cross-reactions in sheep serum against vaccine strains and pathogenic strains of *Brucella*, as well as validation of the agglutination results with ELISA using both vaccine and wild strains of *Brucella*. The used vaccine and wild strains were *RB51, Rev1, B. melitensis*, and *B. abortus*, all of which were confirmed by PCR test. A total of 81 sheep serum samples were included in the study, encompassing both positive and negative reactions in the agglutination tests. Following the characterization of the sera, indirect ELISA tests utilizing the lipopolysaccharide of the bacteria were employed to validate the agglutination results and to statistically assess the serum cross-reactions against the vaccine and wild strains of *Brucella*. Out of 81 evaluated suspected serum samples, the Rose-Bengal test yielded 61 positive and 20 negative results. The Wright and Wright-2ME tests revealed 38 and 36 positive, and 43 and 45 negative results, respectively. According to the ROC curve analysis, the highest area under the curve was 84% for IRIBA, 75% for Rev1, 70% for *B. melitensis*, and 68% for *B. abortus*. Statistical analysis indicated that the strain of antigen in ELISA tests had a significant effect on S/P values. The mean and standard error of the S/P values were as Rev1 vaccine 0.54 ± 0.03, *B. melitensis* 0.82 ± 0.02, IRIBA vaccine 0.82 ± 0.04, and *B. abortus* 0.98 ± 0.04. The highest sensitivity and specificity were achieved using *B. melitensis* and IRIBA antigens, respectively. The indirect ELISA utilizing both vaccine and wild strains of *Brucella* demonstrated appropriate sensitivity and specificity, suggesting its cross-reactions and potential as a reliable diagnostic method for vaccinated and infected sheep.

Key words: Brucella, Sheep, Vaccine, Cross-reaction, ELISA

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