Comparison of two competitive enzyme-linked immunosorbent assays for *Anaplasma marginale* in cattle

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Introduction

Bovine anaplasmosis, caused by *Anaplasma marginale*, is the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable production in the U.S. Several serological assays such as complement fixation (CF), card agglutination, and competitive enzyme-linked immunosorbent assay (cELISA) have been used in the detection of anaplasmosis carriers. The CF and card agglutination tests are not considered reliable due to low diagnostic sensitivities (<2 0% and 67%, respectively). Commercially available major surface protein-5 (MSP-5) epitope-based cELISA is more reliable with high sensitivity (99%) and specificity (89%). Recently, maltose binding protein included as fusion protein in the recombinant MSP-5 used in the commercially available cELISA was identified as the source of some false-positive results. A new cELISA test was developed to improve diagnostic specificity by reducing false positive reactions due to maltose binding protein antibodies and other non-specific antibodies in bovine sera. The objective of this study was to compare results generated using the current and new cELISA tests and real-time RT-PCR to provide veterinarians with up to date information regarding the most appropriate test to use for anaplasmosis diagnosis.

Materials and Methods

Blood was collected from 282 adult beef cows consigned to slaughter plants in the southern U.S. Serum was harvested and then analyzed for anaplasmosis using a commercial competitive enzyme-linked immunosorbent assay (cELISA; Anaplasma Antibody Test Kit, VMRD, Inc., Pullman, WA, USA) and a new cELISA test (VMRD, Inc., Pullman, WA, USA) in accordance with the method recommended by the manufacturer. A confirmatory RT-PCR assay was performed on each blood sample. An *A. marginale*-specific real-time RT-PCR assay was used on RNA extracted from each of the blood samples to detect and quantify a highly conserved and specific region of 16S ribosomal RNA subunit. Sensitivity, specificity, and positive and negative predictive values for both cELISA tests were calculated based upon real-time RT-PCR assay results being the 'true' positives and negatives.

Results

Of the 282 blood samples collected 28 were positive for *A. marginale* by real-time RT-PCR assay for a prevalence of 9.9%. The calculated sensitivity, specificity, and positive and negative predictive values at a prevalence of 9.9% were 85.7%, 96.1%, 70.6%, and 98.4%, respectively for the current cELISA and 82.1%, 96.8%, 74.2%, and 98.0%, respectively for the new cELISA. The degree of agreement of the new and current cELISA with real-time RT-PCR results were both 0.75.

Significance

At a prevalence of approximately 10% the current and new cELISA for diagnosing *A. marginale* may have similar sensitivity, specificity, positive and negative predictive values and agreement with real-time RT-PCR. These results are in disagreement with previous research that indicated the new cELISA had a greater diagnostic specificity than the current cELISA. These results should be validated at different prevalence rates and with multiple reference assays.