Evaluation of Diagnostic Tests for Johne's Disease Using Detection of *Mycobacterium avium* Subspecies *paratuberculosis* in Tissues to Classify Infection Status of Dairy Cows

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Introduction

Interpretation of Johne's disease (JD) diagnostic tests is not always clear to determine the infection status of animals. Multiple tests on an individual animal are often not in agreement. The objectives of this study were to determine the association between infection status of individual dairy cows, based on postmortem histopathology and culture of multiple tissues, and the previous results of fecal culture and multiple serum ELISA tests.

Materials and Methods

Twenty adult dairy cattle potentially infected with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) based on previous serum ELISA and/or fecal culture results were obtained from producers in the Front Range of Colorado. Blood and feces were obtained immediately prior to euthanasia. Serum was separated and frozen until sample collection for the study was complete. Duplicate samples were tested in a single run on one IDEXX ELISA plate to eliminate between-run variation. Mean sample-to-positive (S/P) values were used to categorize cows using the interpretation chart developed by Collins (1998). Complete necropsies were performed on all animals to evaluate any gross lesions and collect multiple tissues for histopathological evaluation and MAP culture. Representative samples of ileum, jejunum, mesenteric and ileocecocolic lymph nodes were evaluated histologically. Samples were evaluated for cellular infiltrates and presence of acid-fast bacteria. Sections of ileum, mesenteric and ileocecocolic lymph nodes and feces were cultured with a radiometric culture technique (BACTEC). Animals with one or more tissues, or feces culture positive for MAP, or evidence of acid-fast bacteria and histological lesions typical of JD, were classified as infected. Previous ELISA results were used to evaluate ELISA variation over time. Proportion of infected animals detected by serum ELISA and fecal culture was determined using results of tissue evaluation and culture as a reference.

Results

Culture results from ten cows were available for comparison, with culture results pending for the remaining ten cows. Histopathologic evaluation of tissues and IDEXX serum ELISA testing have been completed for all cows. Nine of ten cows had one or more tissues and/or feces culture positive and were classified as infected. Histological evidence of MAP infection was not found in tissues of one cow that cultured positive; otherwise, culture and histopathology were in agreement as to infection status. Ten cows were classified as diagnostic, three cows were suggestive and seven cows were shown to have no evidence of MAP infection based on histopathology. Seventeen cows had previous serum ELISA results (initial ELISA) ranging from suspect to strong positive. Days from previous to final ELISA sampling ranged from 66-882, with a mean of 456 and a median of 525 days. Final ELISA S/P ratios varied, with nine cows changing status to a lower category than the initial ELISA, three cows categorized in a more positive category, and five initial strong-positive category cows remaining strong positive based on results of the final ELISA. The proportion of cows that tested suspect or higher with the final serum ELISA and were classified as infected was 100% (9/9), with one cow that had tested positive on the initial ELISA and strong positive on the final ELISA having no evidence of MAP infection. Fecal cultures identified 56% (5/9) of infected cattle.

Significance

At this point in our study, the serum ELISA test has detected a larger proportion of infected animals than fecal culture. Current standards for many state JD control and status programs allow fecal culture confirmation of infection in animals that test ELISA positive. Preliminary results from this study suggest that ELISA-positive, fecal culture-negative cattle may be infected with MAP.