Evaluation of the milk ELISA to determine herd status for Johne's Disease

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Introduction

Results from individual cow milk ELISAs can be used to determine herd status for *Mycobacterium avium* subsp *paratuberculosis* (MAP). Milk samples for the MAP ELISA are less invasive to collect than fecal samples for MAP culture. Moreover, MAP results from the milk ELISA can be obtained quicker and at less expense than MAP results from the culture of feces. The objective of this study was to evaluate the test characteristics of the milk ELISA for determination of herd MAP status in Atlantic Canadian dairy herds. It was expected that many of the MAP-positive herds in this region had low prevalence, and it was of particular interest to evaluate the performance of the milk ELISA within these low-prevalence MAP herds.

Materials and Methods

A total of 32 purposively selected herds from the Maritime provinces participated in this two-year project. Median herd size was 66 milking cows (range, 30 to 220). Individual fecal and milk samples were collected from all milking cows biannually. All fecal samples were processed by the Maritime Quality Milk Laboratory, Atlantic Veterinary College, Prince Edward Island, Canada. Individual cow fecal cultures (grouped by cow age) were combined into pools of five and were cultured using the ESP® Culture System II (TREK Diagnostic Systems, Inc., Cleveland, Ohio, USA). Fecal samples were prepared and cultured in accordance with the manufacturer's instructions. After culture, all broth samples were examined microscopically for the presence of MAP with an acid-fast stain. For all samples that tested positive for MAP by culture or microscopic visualization, confirmatory PCR for detection of IS900 was performed by use of the VetAlert™ Johne's Real-Time PCR kit (Tetracore, Inc., Rockville, MD, USA). Milk samples were tested for the presence of MAP antibodies using three different ELISAs: Parachek2 *Mycobacterium paratuberculosis* Test Kit®, Prionics (ELISA A); *Mycobacterium paratuberculosis* Antibody Test Kit®, IDEXX (ELISA B); and Paratuberculosis Indirect®, IDVet Innovative Diagnostics (ELISA C). Milk samples were processed in accordance with the ELISA manufacturers' instructions. Statistical analysis to estimate the test characteristics of each ELISA were performed via GEE logistic models with exchangeable correlation structures, to account for the repeated measures.

Results

A herd was considered positive by fecal culture (FC) if there was ≥ 1 MAP-positive fecal pool and positive by ELISA if there were ≥ 2% MAP-positive milk ELISA results in the herd. On the basis of FC results, 14 of 32 were considered MAP-positive, and 21, 12, and 18 herds were considered MAP-positive on the basis of results for ELISA A, ELISA B, and ELISA C, respectively. Mean within-herd prevalence (WHP) was 4.6%, 1.9%, 1.4%, and 2.0% for FC, ELISA A, ELISA B, and ELISA C, respectively. Sensitivity, relative to the whole-herd FC, at the herd level was 59.0% (95% confidence interval [CI], 36.2% to 78.4%) for ELISAs A, 55.7% (95% CI, 32.5% to 76.7%) for ELISA B, and 63.3% (95% CI, 40.7% to 81.2%) for ELISA C. Specificity, relative to the whole-herd FC, at the herd level was 80.5% (95% CI, 71.0% to 87.5%) for ELISA A, 95.8% (95% CI, 88.7% to 98.5%) for ELISA B, and 91.7% (95% CI, 86.5% to 95.7%) for ELISA C. For model development, the remainder of the analysis focused on results from ELISA B only. The MAP WHP as determined by FC was a significant predictor in the null model Se for ELISA B; the herd-level Se of ELISA B increased as FC WHP increased. For example, when FC WHP was 1%, the herd-level Se of ELISA B was 11.4% (95% CI, 3.0% to 35.2%) and when FC WHP was 6%, the herd-level Se of ELISA B was 75.2% (95% CI, 44.3% to 92.1%). A GEE model was used to predict FC WHP on the basis of a transformation of the milk ELISA WHP values. The relationship is best displayed graphically, but as examples, when ELISA WHP was 1%, the FC WHP was estimated to be 0.7% (95% CI, 0.4% to 1.2%) and when ELISA WHP was 7%, the FC WHP was 9.4% (95% CI, 7.8% to 11.4%). Likelihood ratios (LR) based on four categories of milk ELISA WHP values were developed. Essentially, a herd with a milk ELISA WHP of 0% was one-quarter as likely to be MAP-positive as MAP-negative (LR 0.25), herds with milk ELISA WHP values of > 0% to 2% had a similar likelihood to be FC-positive or FC-negative (LR 1.17), and herds that had a milk ELISA WHP of > 2% to 4% were more than three times as likely to be MAP-positive than MAP-negative

(LR 3.33). All herds that had an ELISA WHP > 4% were FC-positive; therefore, a LR could not be defined.

Significance

Both Se and Sp of the milk ELISA at the herd level are variable. When a cut-off of ≥ 2% positive milk ELISAs was required to designate a herd as MAP-positive, the imperfect Sp frequently led to false-positive herd diagnoses. The Se was particularly affected by the FC WHP, and in herds with a low FC prevalence (≤ 5%), the herd-level Se of the milk ELISA was low, making false-negative diagnoses frequent. Although the milk ELISA worked well to positively identify herds with MAP-infected cows when the FC WHP is > 10%, in low-prevalence herds interpretation was a challenge. When the milk ELISA was applied at the herd level, if there were > 0% but < 2% of cows testing positive, the likelihood of that herd being MAP-positive versus MAP-negative was essentially equivalent.