

# Evaluation of a Milk ELISA for Bovine Paratuberculosis

Michael T. Collins, DVM, PhD, Dipl. ACVM

Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, WI 53706-1102

## Introduction

Johne's disease, caused by *Mycobacterium paratuberculosis*, has become a prevalent infectious disease problem for dairy cattle herds. Control programs require changes in herd management to limit opportunities for infection transmission to young stock, coupled with diagnostic testing to identify the infected, or at least most infectious, adult cattle. When within-herd infection rates are high, it is not economically feasible to cull all test-positive cows. Consequently, it is necessary to adopt testing strategies that provide both diagnostic and prognostic information. The owner needs to know which cows are most infectious and are unlikely to survive another lactation; these cows need to be removed from the herd. It also would be helpful to know which infected cows are least infectious and are capable of sustaining another lactation and generating farm income. Veterinary diagnostics for food animals are strongly affected by end-user economics. Consequently, the most accurate and informative test results must be provided to the end users at the least cost. The diagnostic technology fulfilling this need is often based on antibody detection using enzyme-linked immunosorbent assay (ELISA) technology because of its low cost and high throughput potential through automation. Application of ELISAs to milk, instead of blood, samples brings even further efficiency. The purpose of the present study was to evaluate the sensitivity and specificity of a commercially available milk ELISA for Johne's disease and evaluate its cost-effectiveness using an economic decision analysis model.

## Materials and Methods

Milk ELISA (AntelBio Systems) accuracy was evaluated using milk samples from 352 dairy cattle in seven paratuberculosis-free herds (status level 4 of the US Voluntary Bovine Johne's Disease Herd Status Program) and 2,094 dairy cattle in seven *M. paratuberculosis*-infected dairy herds. Three independent laboratories using three different culture procedures completed fecal cultures for *M. paratuberculosis* on these cattle and found 417 cows to be shedding *M. paratuber-*

*culosis* in their feces. All cattle in the seven status-4 herds were considered free of *M. paratuberculosis* infection, and thus used for assay specificity estimation. Cattle that were fecal culture-positive for *M. paratuberculosis* by any of the three laboratories were considered confirmed cases of infection and used for assay sensitivity estimation.

## Results

Sensitivity and specificity of the milk ELISA were 28.85% (95% CI; 26.5% - 31.2%) and 99.72% (95% CI; 99.43% - 100.0%), respectively. These accuracy parameters were comparable to those of two serum ELISAs evaluated simultaneously. Also, milk ELISA results had a high level of agreement with the two most widely used commercial serum ELISAs (kappa 0.66 and 0.68) for categorical assay interpretations (positive or negative), although linear regression of quantitative results showed low correlation coefficients ( $r^2 = 0.38$  and  $0.56$ ) due to the fact that ELISA results for some cows were high in one assay but low in another assay. Likelihood ratio analysis showed a direct relationship between the magnitude of milk ELISA result and the odds of a cow shedding *M. paratuberculosis* in its feces. When test accuracy and test costs for five commonly used tests for paratuberculosis were used in a decision analysis model (described in another American Association of Bovine Practitioners presentation), milk ELISA was the test most often recommended as having the best cost-benefit.

## Significance

If used judiciously and interpreted quantitatively, milk ELISA is an accurate and cost-effective tool in support of paratuberculosis control programs in dairy herds. These results, however, only apply to the test as performed by the AntelBio. Because the milk ELISA is an in-house assay (not an ELISA kit licensed by the USDA), it is hazardous to assume that a milk ELISA performed at other laboratories will have the same performance characteristics.