

# Diagnosis of Johne's disease – A new tool for MAP serology

Joanna Rzepka, BS, PhD; Siddra Hines, BS, DVM, PhD, DACVIM  
VMRD Inc, Pullman, WA 99163

## Introduction

Johne's disease is a chronic and highly contagious wasting disease of all ruminants caused by *Mycobacterium avium ssp paratuberculosis* (MAP). Major clinical signs include weight loss, pipestream diarrhea, edema, decreased milk production, and eventually death. In the US, Johne's disease affects ~70% of dairy herds, costing the industry up to \$250 million per year in production loss. An estimated 5 to 10% of beef herds are also affected. Johne's disease represents a significant problem in goat and sheep herds as well; however, no serologic tests are currently licensed by the USDA for small ruminants.

## Materials and Methods

An indirect ELISA was developed based on antigen processed from whole MAP, with an anti-ruminant secondary antibody to target detection of cattle, sheep, and goat anti-MAP antibodies. Serum and milk samples from 13 farms were acquired from the Johne's Disease Integrated Program, with MAP prevalence of 0% for 2 farms and 2 to 27% for the remaining 11 farms based on fecal culture at the time of sample collection. All samples were characterized based on fecal culture and PCR, with 243 negative and 61 positive serum samples and 91 positive and 98 negative milk samples. An additional 372 culture and PCR positive samples with no herd history were obtained, 329 from Dr. Michael Collins at the University of Wisconsin and 43 from the National Veterinary Services Laboratory, for a total of 433 positive samples. Each sample was tested in the VMRD MAP Ab ELISA kit with a sample:positive ratio cutoff of 0.3. Results of 2 other commercial ELISA tests were also available for comparison. For preliminary assay validation in goats, the negative cohort consisted of 76 negative serum samples collected from a Johne's-free herd, and 10 other confirmed negative samples

from the sham vaccinated group of a Johne's vaccination study. From this same study, 71 test-positive samples were obtained for the positive cohort.

## Results

Sensitivity of this assay for detection of anti-MAP antibodies in serum samples from cattle positive for Johne's on fecal culture and PCR was 93.1%, and specificity was 90%. In comparison, 2 other commercial ELISAs demonstrated sensitivity of 91% with 85.4% specificity, and 86.6% with 89.3% specificity with the same sample set. For milk samples, sensitivity and specificity were 72.5% and 88.8%, respectively, compared to 64.8% and 86.7% for another commercial ELISA validated for bovine milk. With a specificity of 100%, sensitivity of this assay for detection of anti-MAP antibodies in goat serum samples was 72.5%. Testing of this same sample set in 2 other commercial ELISAs demonstrated sensitivity of 62.9% and 52.9% at a specificity of 100%.

## Significance

Diagnosis of Johne's disease in any species poses unique challenges due to the nature of the bacterium and its interactions with the immune system as well as the prolonged course of disease. Currently available technology limits the sensitivity of serology in all species; however, serology remains a cost-effective method for herd surveillance. Proper diagnostic testing is pivotal for management efforts as efficacious vaccine or specific treatments are available for any species. This new option for Johne's disease serology has been submitted for USDA licensure for bovine serum, and additional uses are currently being investigated, including bovine milk, caprine serum, and ovine serum.