

# Evaluation of the Sensitivity of Three ELISA Tests for the Detection of *Mycobacterium avium paratuberculosis* Infection using Tissue Culture as a Gold Standard

Shawn LB McKenna, BSc, DVM<sup>1</sup>; Greg P Keefe, DVM, MSc, MBA<sup>1</sup>; Donald C Sockett, DVM, PhD<sup>2</sup>; John A VanLeeuwen, DVM, PhD<sup>1</sup>; J McClure, DVM, MSc<sup>1</sup>; Paul Hanna, DVM, PhD<sup>1</sup>; E Spangler, DVM, PhD<sup>1</sup>; Herman W Barkema, DVM, PhD<sup>1</sup>

<sup>1</sup>Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada

<sup>2</sup>Wisconsin Veterinary Diagnostic Lab, Madison, Wisconsin, USA

## Introduction

The sensitivity of enzyme linked immunosorbent assay (ELISA) techniques for detection of cattle infected with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) has been reported to be between 15 and 75%. Nearly all sensitivity evaluations of these ELISAs have utilized fecal-culture positive animals as the gold standard. This approach may result in inflated estimates of sensitivity, because animals in early stages of infection may not shed the bacteria consistently in their feces. The purpose of our study was to establish an estimate of the sensitivity of three ELISA tests for MAP, using sera from dairy cattle that were tissue-culture positive.

## Materials and Methods

Culled dairy cattle (n=994) were selected for enrollment in a study using a systematic random process at a regional abattoir. Samples were collected weekly over a 10-month period. Animals were sourced from northern New England (n=131) and Atlantic Canada (n=863). Serum, terminal ileum, and two mesenteric lymph nodes (ileo-cecal region) were collected from each animal.

The serum was tested with three different ELISAs, including two currently used in North America, the IDEXX Herdchek<sup>®</sup> ELISA (IDEXX Laboratories Inc, Westbrook, ME) and the Biocor Parachek<sup>™</sup> ELISA (Biocor Animal Health, Omaha, NE). A third investigational ELISA was also tested, the SVANOVIR<sup>™</sup> Para-TB-Ab ELISA (Diagnostic Chemicals Limited, Oxford, CT). The first two assays were run at the Wisconsin Veterinary Diagnostic Laboratory and the third was tested in Uppsala, Sweden. Each tissue was cultured using the VersaTREK<sup>™</sup> broth media (TREK Diagnos-

tic Systems Inc, Cleveland, OH). The broth culture was examined microscopically after acid-fast staining (Ziell Nielson), and all suspicious samples were confirmed with subculture on Herrold's egg yolk media.

## Results and Conclusions

In total, 160 animals (16.1%) were MAP positive on culture. The IDEXX Herdchek<sup>®</sup> ELISA classified 14 of the 160 animals as positive for a sensitivity of 8.75% (95% CI = 4.37, 13.12). The Biocor Parachek<sup>™</sup> ELISA classified 11 of the 160 animals as positive, for a sensitivity of 6.88% (95% CI = 2.95, 10.8). The SVANOVIR<sup>™</sup> Para-TB-Ab ELISA classified 27 of the 160 animals as positive, for a sensitivity of 16.88% (95% CI = 11.07, 22.68). The IDEXX and Biocor ELISA sensitivities were not statistically different from each other, but the SVANOVIR<sup>™</sup> ELISA had a statistically significantly higher sensitivity.

The estimates of sensitivity found in this study are lower than estimates previously described in the literature,<sup>2</sup> however, they are similar to an ELISA sensitivity (15.4%) reported in a recent study using low fecal shedders.<sup>1</sup> The lower sensitivity found in this study may be due to identification of infected animals in the earlier stages of disease.

## References

1. Dargatz DA, Byrum BA, Barber LK, Sweeney RW, Whitlock RH, Shulaw WP, Jacobson RH, Stabel JR: Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. *J Am Vet Med Assoc* 218(7):1163-6, 2001.
2. Sockett DC, Conrad TA, Thomas CB, Collins MT: Evaluation of four serological tests for bovine paratuberculosis. *J Clin Microbiol* 5:1134-9, 1992.