Interpretation of Repeated Testing for Johne's Disease in Ontario Dairy Herds.

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Introduction

The Ontario Johne's Education and Management Assistance Program (OJEMAP) is a dairy industry funded Johne's Disease (JD) control program launched in January 2010. The program offers dairy producers a one-time opportunity to test their adult cow herd for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) antibodies using a milk or blood ELISA test.

As herds completed their milking herd test, a common question from herd owners and veterinarians was "When should I test next….how often should I test my herd?" A review of aggressive JD control programs around the world suggests that there are no standard recommendations regarding testing frequency. The Danish program, in existence for seven years, is based on quarterly testing of the entire milking herd using a milk ELISA. Dr. Soren Nielsen, who developed the Danish Program and serves as its director, argues that given the relatively poor sensitivity of all JD tests, quarterly testing allows each cow to have at least three test results per year, the results of which can then be used to classify cows as high, moderate or low risk for spreading MAP to herd mates. Implementation of this strategy has lead to a decrease in JD test-positive prevalence from 10% to 6% of cows in herds participating in the program. Unfortunately, this aggressive herd test program carries a high cost and the benefits of quarterly testing over a single annual test are difficult to quantify.

The objective of this project was to evaluate and gain experience in the interpretation of repeated testing for JD in Ontario dairy herds, using JD tests currently available in Ontario.

Methods

Through the OJEMAP 10 herds with test positive cows and active Johne's control programs were identified for participation in this project. These 10 herds were scheduled to be tested quarterly for 18 months (six tests in total), using the Johne's milk ELISA offered by CanWest DHI (previously the Prionics Milk ELISA). With an average herd size of 90 milking cows, the test results from this project were expected to generate a dataset containing multiple test results from over 900 unique cows. These data were used to classify cows as consistently test negative (CNEG - all test results below the 'suspect' threshold of 0.07 SP), consistently test positive

(CPOS - once a cow had a positive test result, all subsequent tests were positive), or variable test results (VAR - at least one positive test result followed by at least one negative test result).

Results

All of the study herds have completed five full herd tests, with a final herd test pending. The majority (982) of the 1,120 unique cows were CNEG, while 111 were CPOS and 27 were VAR.

Thirteen of the 27 VAR cows had a sequence of at least three positive tests, with one negative test embedded in that sequence. For all 13 the negative test followed the first positive test (P,N,P,P). All subsequent tests were positive and most demonstrated an increasing SP ratio over time. The 13 cows are of particular interest as they present a contradiction at their second test (the negative test after a positive test), suggesting that the first test result may have been a *false positive*. By following these cows over time, it is clear that in these 13 cases their subsequent tests are also positive, and from that point onward, their antibody levels continue to rise, suggesting that the first test may have been a transitional result as the cow began to mount an antibody response to MAP. This has significantly increased our confidence in the use and interpretation of the milk ELISA. Three additional cows had a positive test, followed by a negative test as their only two tests to date. We are awaiting further test results to learn if these two will also follow the pattern described for the preceding 13 cows.

Nine cows had a series of positive and negative test results, all very near the 0.1 SP cut-point, suggesting that these cows were producing antibody at continuously low levels, near the cut-point, and did not follow the pattern of increasing antibody production over time.

The final two cows had a single moderate to high test value followed by one or more negative tests. These positive tests may well have been false positive results.

Based on repeated testing we have also identified cows in higher prevalence herds that seem to be expressing different patterns of disease progression based on antibody response. For example, typically cows develop antibody over a short period of time, the antibody levels increase until they reach high positive levels and some of these cows go on to develop clinical disease. The interesting observation is that in some cases we see this pattern develop in young cows (2 or 3 years old), while in other instances it doesn't happen until they are older (6 or 7 years old). It will be important to study these individuals in more detail to determine whether the delay in antibody production is a function of the cow, the organism, the infective dose or whether delayed or repeated exposure to MAP plays a role.

Significance

While JD is a slowly progressive disease, the dynamics of infection and herd prevalence need further study. The repeated test results from these 10 herds yielded consistently negative or positive results for 98% of the adult milking cows. Careful examination of the series of test results from the 27 cows with variable test values reminds us that antibody concentrations in

milk will vary over time, and may fluctuate around the test positive/negative cut-point, in some cases producing perceived false-positive or false-negative results. Given this knowledge, we must be cautious about condemning or retaining cows based on a single MAP milk ELISA test, especially when positive values are near the positive/negative cut-point.