Interpretation of repeated testing for *Mycobacterium avium* subsp *paratuberculosis* on 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 antibodies against *Mycobacterium avium* subspecies *paratuberculosis* (MAP) by use of 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 7 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 3 test results per year, the results of which can then be used to classify cows as at high-, moderate-, or low-risk for transmitting MAP to herd mates. Implementation of this strategy has led 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 MAP with currently available tests in Ontario dairy herds.

Materials and Methods

Through the OJEMAP database, 10 herds with MAP test-positive cows and active JD control programs were identified for participation in this project. These 10 herds were scheduled to be tested quarterly for 18 months (6 tests in total) with the MAP 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 [S/P ratio, 0.07]), consistently test-positive (CPOS - once a cow had a positive test result, all subsequent tests were positive), or variable test results (VAR - at least 1 positive test result followed by at least 1 negative test result).

Results

At the time this abstract was submitted, all of the study herds had completed 5 full herd tests, with a final herd test pending. The majority (n = 982) of the 1,120 unique cows were CNEG, whereas 111 were CPOS, and 27 were VAR.

Thirteen of the 27 VAR cows had a sequence of at least 3 positive tests, with 1 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 S/P ratio over time. These 13 cows are of particular interest because 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 for these 13 cows, their subsequent tests are also positive, and from that point onward, their antibody titers continue to rise, suggesting that the first test may have been a transitional result as the cow began to mount an antibody response against 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 2 tests to date. We are awaiting further test results to learn if these 2 will also follow the pattern described for the preceding 13 cows.

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

The final 2 cows had a single moderate to high S/P ratio followed by one or more negative tests. These positive tests may well have been false positive results.

On the basis of repeated testing we have identified cows in herds with high within-herd JD prevalence that express different patterns of antibody response against MAP. For example, typically cows develop antibodies over a short period of time, the antibody titers increase until they reach high-positive levels, and some of these cows go on to develop clinical JD. The interesting observation is that, in some cattle, we see this pattern develop in young cows (2 or 3 years old), while in other cattle it does not develop until they are older (6 or 7 years old). It will be important to study these individual cattle in more detail to determine whether the delay in antibody production is a function of the cow, the organism, or 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 results reminds us that antibody concentrations in milk vary over time, and may fluctuate around the test-positive cut-point, which can result in perceived false-positive or false-negative results. Given this knowledge, we must be cautious about condemning or retaining cows based on the results of a single MAP milk ELISA test, especially when positive values are near the test-positive cut-point.

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