DAIRY VETERINARY NEWSLETTER

September 2022

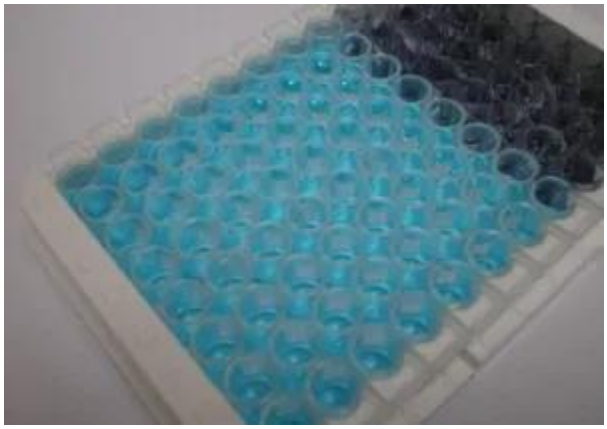
How does Comparative Cervical TB Testing Affect Johne's disease Milk ELISA Tests?

After nearly 90 years of attempting eradication in the U.S., bovine tuberculosis (TB) continues to be greatly reduced in prevalence, but still not quite eradicated. Along with brucellosis testing, TB testing is one of the primary zoonotic disease monitoring programs in the dairy industry not only in the U.S. but in much of the developed world. Even in European countries that have declared bovine TB eradicated for 40 to 60 years, there is still surveillance for the disease. In the U.S., bovine (and caprine and ovine) TB testing is first performed using the caudal fold intradermal tuberculin test that nearly all accredited veterinarians are quite familiar with.

According to the USDA Animal and Plant Health Inspection Service (APHIS) current regulations, "The [caudal fold] test must be read between 66 and 78 hours after injection (72 hours is optimum). The veterinarian who made the injection must be the veterinarian who reads the test result. - - Visually inspect the injection site closely and palpate it carefully to detect changes from the normal. Any swelling, sensitivity, or increase in thickness of the skin is considered to be a positive response to the tuberculin. The size of responses may vary and are not indicative of infectious status. Responses may be small, hard, pea-sized responses, diffuse responses, circumscribed responses, or large responses. If there is doubt about whether a response has occurred, palpate the opposite side of the tail to determine if there is a change from normal." The test results are considered Suspect "when you observe or palpate any increase in caudal fold thickness, size, or sensitivity, at the injection site as described above." In the case of animals with a Suspect reaction, "- - immediately notify your APHIS - VS District Office and State animal health officials. The caudal-fold [TB] test is used as a presumptive diagnostic procedure, and animals classified as suspect must be evaluated further by the comparative cervical (CC) test, gamma interferon test, or sent directly to slaughter under permit." The CC test is still commonly performed on suspect cattle. The primary purpose of the test is to differentiate mammalian from avian TB.

Image of the comparative cervical TB test

Johne's disease (JD) is one of the most important dairy production loss diseases, often suspected as zoonotic because it may contribute to the development of Crohn's disease (it is also now reported to be associated with risk of multiple sclerosis as well) in humans. Before diarrhea and emaciation eventually result in many infected cattle, milk production loss and therefore marked increase in culling are commonly observed. As we have reported previously in this newsletter and in refereed publications, the JD milk ELISA test is virtually identical to fecal testing for detection of JD in lactating dairy cows. A paper by E. Nunney et al. in the Journal of Dairy Science, October 2022 examines the question of how the CC test affects the results of the JD milk ELISA test. In the introduction section, the paper states that in comparison to fecal culture, " the ELISA is relatively low cost, more convenient, and faster." (The paper also describes an interesting "green," "yellow," or "red" JD status classification scheme used in the U.K. based on repeated milk JD ELISA testing.) The entire paper can be found at: https://www.journalofdairyscience.org/action/showPdf?pii=S0022-0302%2822%2900490-8



Most DHIA and other milk testing laboratories can readily send milk samples for further testing by the JD Milk ELISA test; test wells shown above

The Nunney paper states that previous research has found that an "increase in [anti-JD] antibodies post [CC] has been shown - - however, it is not clear whether this rise leads to an increase in sensitivity due to a potential anamnestic [booster] effect from the [purified protein derivative used in CC] in JD-infected animals or due to antibody cross-reaction in JD-noninfected animals or a mixture of both." (More on this will follow later,) The authors also note that "there have been anecdotal reports that [JD] testing soon after [CC] testing may help identifying [JD] infected cows. A recent study - - showed an increase in the odds of a positive [JD] test result when [JD] milk ELISA testing occurs less than 30 [days after CC] testing."

The objectives of the study were to "determine the effect of time since [CC] testing on [JD] milk ELISA test values - - for JD-infected and noninfected cows, and to estimate the effect of testing interval between [CC] and [JD] testing on the capacity of the [JD] milk ELISA to correctly classify cows according to their [true JD] infection status."

The authors had access to a massive data set for the study. After excluding duplicate test results and cows with extremes of age (< 20 months old, > 20 years old), there were nearly 10 million JD milk ELISA (IDEXX Paratuberculosis Screening Ab Test) tests performed on over 1.6 million cows in the U.K. Most cows were sampled every 3 months during lactation. A definition of "true" status for JD was that within 4 consecutive JD milk ELISA tests, cows with at least 2 positive results (2/4, 3/4, or 4/4 +) were true positives, truly infected with JD, while cows with 0 or 1 positive test result (0/4 or 1/4 +) were true negatives, truly uninfected with JD.

The authors decided that no cow would be classified as truly infected with JD if one of the critical positive JD tests was within 90 days after CC. (E.g. if a cow was 2/4 + but one or both positive tests was ≤ 90 days after CC, she was excluded.) This excluded 14% of all cows that would have been defined as JD +. However, among the

86% of the JD + cows that still qualified for the study, many cows had at least one other JD test within 90 days after a CC test. There were 66,156 JD + cows and 739,405 uninfected cows from January 1, 2012 to September 29, 2018. The cows came from 3226 herds. It has been quite a few years since I have seen this with the increased computation capacity of computers today, but reducing statistical computation time was important in this study, therefore "one [and only one] observation from each noninfected cow and infected cow was randomly selected."

Cows were divided into 4 categories of the time since CC testing when a JD milk ELISA was performed: 0 to 14 d, 15 to 28 d, 29 to 60 d, 61 to 90 d. The 61 - 90 d group was used as baseline; the likelihood of a positive JD test in the other groups was compared to that. Generalized additive mixed models with complex statistical methods including a gamma logarithmic link function are described in the full paper. Age of cows in days was also included. Some of the methods I had never heard of before. To me, it was somewhat disappointing that because only one test result from each cow was used, a simulation was run 1,000 times to calculate sensitivity and specificity of the milk ELISA. Another simulation was run 10,000 times to calculate "mean accuracy [sensitivity and specificity] and predictive values" of a positive and negative JD milk ELISA result.

Results

From the true JD + cows, 47% of all ELISA tests were positive; from the true JD - cows, 2% of tests were positive. Median lactation number of all cows, and also the median lactation number when they were first detected JD + was 4th lactation. Among JD infected cows, the increased likelihood of a JD + ELISA result for the time intervals since CC testing was: 29 - 60 d, 0; 15 - 28 d, 25%; 1- 14 d, 36%. However, for uninfected (true JD -) cows, the increased likelihood of a false JD + result was: 29 - 60 d, 15%; 15 - 28 d, 235% (3.35 x); 1- 14 d, 213% (3.13 x).

Similar to the above results, the effects of CC on JD ELISA sensitivity and specificity were virtually zero as long as JD testing took place at least 30 d after CC testing. In close agreement to many previous studies, the JD sensitivity (detection of true positives) was: 29 - 60 d, 54%; 61 - 90 d, 53%. Specificity (avoiding false positives) was: 29 - 60 d, 98%; 61 - 90 d, 98%. Testing for JD is a classic example of disease screening where high specificity is nearly always critically important, and the resultant confidence in any positive test result means that repeated testing over time is very useful, and compensates for the lower sensitivity on any one test. (E.g. a cow whose JD milk ELISA results over time are + - - + - or even + + - - is virtually certain to be truly infected. Intermittent shedding in both milk and/or feces is a hallmark of JD.) Again, this is because false positive results are so uncommon when we set the thresholds for a positive ELISA result the way we usually do (high specificity).

We should always keep in mind that predictive values of both a positive test (PV +), the % of all positive tests that are correct (the % of all positive tests that are in true JD infected cows) and of a negative test (PV -), (the % of all negative tests that are in cows truly uninfected with JD) are completely influenced by the prevalence of the disease in the cow population sampled. The PV statistic is often misleading and misused. However, with a prevalence of 5% of a herd infected with JD, the PV values were: PV +: 29 - 60 d, 54%; 61 - 90 d, 53%, and PV -: 29 - 60 d, 97%; 61 - 90 d, 97%. With 20% of the herd infected with JD, PV values were: PV +: 29 - 60 d, 84%; 61 - 90 d, 84%, and PV -: 29 - 60 d, 88%; 61 - 90 d, 87%. Again, there was virtually no effect on PV's as long as JD testing took place at least 30 d after CC testing.

There is no conclusive evidence regarding the mechanism of the effects of CC TB testing on JD milk (or serum) ELISA results. It is likely that the similarity of *Mycobacterium bovis* (TB in ruminants is sometimes caused by other *Mycobacterium* spp. including *M. tuberculosis*) and *Mycobacterium avium* subsp. *paratuberculosis* organisms lead to some cross-reactivity, but that is informed speculation only. The authors' conclusion was, "To avoid interference from the [CC] test in - - cows the milk [JD] ELISA should be evaluated more than 30 d post [CC] testing."

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In practical terms, one important consideration is that when screening lactating cows for export, import, purchase from another herd, or those returning from a heifer raising facility, it would be better to run the JD milk ELISA first, then do intradermal TB testing - in case any CC tests are then needed - instead of having to wait 30 days if CC testing were performed first.

Dairy Convention in Provo, UT January 11 - 12, 2023

Dairy West is organizing the 2023 Dairy Convention (it is no longer called the Utah Dairy Convention because it is an outreach event for Idaho and other states' members of the dairy industry also). It will be at the Provo, UT Marriott Hotel and Convention Center. There will be a dinner on January 11 and presentations on January 12, 2023 to finish by 3:00 p.m.

More details of the program and events will be coming. In the past, some subjects covered have been of interest to dairy veterinarians as well as dairy industry clients.

Please let us know your comments and suggestions for future topics. I can be reached at (435) 760-3731 (Cell), or David.Wilson@usu.edu.

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