TB test interference with JD ELISA - Literature review

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1 Executive Summary

The potential for cross-reactivity in diagnostic testing for mycobacterial diseases including paratuberculosis (Johne's disease, JD) and bovine tuberculosis (TB) has been well recognised, although no trials in a New Zealand context were found in published literature. New Zealand's dairy herds are pasture-based, seasonally calving and comparatively large, and operate in a temperate climate with typically high rainfall. The interaction between TB testing and JD testing on New Zealand farms may be confounded by environmental and management conditions, but the magnitude of such confounding and therefore the external validity of international trials cannot be determined without detailed field studies. A well-designed trial yielding robust and externally valid results would be logistically difficult, expensive and inconvenient in a field situation. The relevance of these international case study findings cannot be easily extrapolated to produce advice for the dairy farmers and vets in New Zealand because of the inherent limitations of case study evidence.

However, assuming the effect of environmental and management conditions is minimal, sufficient evidence already exists in the international literature to justify recommending a time interval of 43 days after TB testing before a JD milk ELISA testing and 71 days before JD serum testing is conducted to avoid non-specific results and unnecessary culling of these false positive animals.

2 Literature review

The family of mycobacteria are well known for cross-reactivity in diagnostic testing in the live animal due to antigenic similarity. Cross-reactions between bovine tuberculosis (TB) and Johne's disease (JD) have been described in the international literature for many years (see for example Brito and Aly, 2014; Dunn et al., 2005; Souza et al., 2020). JD infection has been anecdotally recognised in New Zealand as causing false positive reactivity in tuberculin skin testing (J. Sinclair, OSPRI, pers. comm). Tuberculin exposure at TB testing has likewise been suggested as a possible cause for ELISA reactivity in JD negative animals (Varges et al., 2009; Brito and Aly, 2014) and JD infected animals (Barden et al., 2020; Roupie et al., 2018, Bridges and van Winden 2021). Based on the results of a trial conducted in 2014 on a spring calving herd of 139 cows with 8% seroprevalence (Kennedy et al., 2014), National Milk Laboratories (NML) in the UK advises a minimum 43-day interval between tuberculin injection and JD milk ELISA testing (NML, 2022), although other authors have concluded that the effect of tuberculin exposure may last up to 90 days (Varges et al., 2009). Antibody levels are higher and more persistent in serum than milk, and NML also recommends a minimum 71 days between tuberculin injection and JD serological testing.

In the UK and Europe, active surveillance for bovine tuberculosis involves whole-herd comparative cervical intradermal tuberculin testing, which involves injection of both avian and bovine tuberculin at different sites on the neck to improve specificity over single bovine tuberculin testing (Sedighi & Varga, 2021). In New Zealand, a single intradermal injection of bovine tuberculin is given into the tail fold, with serial bovine gamma interferon testing on whole blood 10-30 days later to increase specificity in the case of a likely false positive animal in a low-risk situation (OSPRI, 2022). Thus, tuberculin testing in the UK involves exposure to both avian and bovine tuberculin, while in New Zealand the exposure is to bovine tuberculin only. In theory, avian tuberculin should induce a greater JD antibody ELISA response than bovine tuberculin, due to its antigenic similarity with *Mycobacterium avium* subsp *paratuberculosis* (MAP), although in one study, cross-reactivity was only seen after administration of a single injection of bovine tuberculin or the comparative cervical test, and not from avian tuberculin alone (Varges et al., 2009).

Testing the hypothesis that bovine tuberculin exposure causes increased JD ELISA reactivity in the New Zealand situation and quantifying the effect of such exposure would be difficult in a field situation for a number of reasons. Antibody levels are known to fluctuate on an individual animal level over a milking season (Navarro-Gonzalez et al., 2018). On a herd level, median test results tend to follow a U-shaped curve, with higher S/P values in early and late lactation (K. Dawson, LIC, unpublished data). Thus, herd- or individual-animal level variations between two consecutive tests with an intervening tuberculin test may be due to natural antibody fluctuations within the season, and not attributable to tuberculin exposure. Additionally, stressful events are known to have an initial suppressive effect on JD antibody titre, followed by increased MAP colonisation and shedding and a resultant rise in antibody levels four to six weeks after the event (K. Bond, NMR, pers. comm). A stressful event such as flooding, drought or extreme heat may therefore confound the relationship between TB testing and JD ELISA results.

JD infected and uninfected cows may be affected differentially by tuberculin exposure. In infected cows, tuberculin exposure may increase sensitivity of detection of JD by stimulating an anamnestic humoral response (Bridges and van Winden, 2021). On the other hand, tuberculin administration prior to a JD ELISA test may result in a decrease in specificity in uninfected cows by enhancing a non-specific response to environmental mycobacteria (Varges et al., 2009). These effects are difficult to separate. Additionally, determining the true JD status on an individual or herd level is difficult, due to the absence of a useful gold standard. Therefore, any field trials would need to be conducted over herds with a wide range of within-herd prevalence for the findings to be applicable to the target population. To estimate the duration of an effect would require repeated sampling of milk for JD antibodies over the first six or more weeks following tuberculin administration in the required number of herds. This would be logistically very difficult in a field situation because of the inconvenience caused to farmers.

3 DISCUSSION AND RECOMMENDATIONS

Case study observations are considered the lowest quality evidence in the epidemiological hierarchy. Anecdotal evidence collected by LIC cannot be used to robustly justify advice to farmers and vets around the timing of testing or the interpretation of unexpected results. The literature however contains several recent and well-designed studies, which can be used as a body of evidence to formulate such advice, even though the trials were not conducted in New Zealand.

There are a few biologically plausible reasons to suggest that the potential for cross-reactivity may be different in New Zealand than in other countries that have reported these findings. New

Zealand's climate and weather conditions, comparatively large dairy herds, prevalence of environmental mycobacterial exposure, all-year pasture-based system and seasonal calving pattern may all have a confounding effect on the association between TB testing and JD ELISA outcome. Without detailed field trials, the magnitude of the effect cannot be assessed, but is unlikely to be more pronounced in New Zealand than in other countries because of its immunological basis. An approach to minimise wastage due to non-specificity would be for LIC to recommend a 43-day interval after TB testing before collection of herd test milk for JD testing, and at least 71 days before JD serum testing.

Because of the difficulty in conducting a well-designed trial under field conditions on New Zealand farms and the low yield of information in comparison to the cost of undertaking this work, further trial work is not recommended. LIC therefore made the decision to take the conservative approach by adopting the UK recommendations for timing of JD ELISA testing post-tuberculin exposure.

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