2016 JDRC Milk ELISA Report

Herd Screening options for Johne's Disease: Evaluation of herd test and Bulk Tank Milk ELISA

1. Introduction

One of the most important goals for a Johne's disease (JD) risk management plan is reducing the risk of transfer of Johne's bacteria *Mycobacterium avium ss. paratuberculosis* (MAP) from older and shedding cows to susceptible young stock. In conjunction with other on-farm management practices, an effective tool to help achieve this is to individually test the whole herd to identify those with JD. Removal of these cows, which are in the pre-clinical but heavy shedding phase, will help reduce the amount of MAP being shed on farm.

The identification of very early subclinical cases is expensive and ineffective due to the nature of infection; as the disease progresses, the bacteria are increasingly shed in faeces and diagnosis of JD becomes easier and more reliable. While a range of tests are available both to detect MAP or the immune response to infection, only PCR (Polymerase Chain Reaction) and ELISA (Enzyme-Linked Immunosorbent Assay) tests are recommended for the routine detection of JD in dairy herds. The performance of ELISA tests improves as the progression of the disease causes antibody levels to rise. In this report, the feasibility of testing herd test milk samples for whole herd screening using a commercial ELISA kit was evaluated.

Herd level screens have also been considered by LIC to determine if a reliable indicator of infection in the herd can be identified. Two options have been evaluated including Bulk tank milk (BTM) screening with ELISA (described below) and real-time PCR for detection of MAP in effluent (described in 2016 JDRC PCR Screening report)

1.1 Aim

1) Evaluate the use of a commercial ELISA kit for testing individual herd test milk samples to identify JD cows by comparing with serum ELISA and faecal culture.
2) Determine the feasibility of bulk tank milk screening ELISA as a herd level JD risk indicator.

2. Material & Methods

2.1 Herd test and bulk tank milk samples

Following herd testing, individual milk samples were transferred from LIC Herd Testing Laboratory to the Animal Health Laboratory. Samples were rearranged from herd test trays to an appropriate testing format using a built-for-purpose consolidation robot (Intellitech Automation; Figure 1A, B).

Subsamples of milk were then aliquoted into 96-well storage plates ready for testing using a Microlab STAR Liquid Handling Workstation (Hamilton; Figure 1C).

Bulk tank milk samples were obtained from MilkTestNZ. Samples were manually rearranged to an appropriate testing format and subsampled into 96-well storage plates as described for herd test milk samples.

2.2 Pooling of herd test milk samples

To minimise the cost of screening the whole herd, an intermediate pooling step (10 herd test samples per pool) was introduced by LIC Animal Health Lab. Pooling of herd test milk subsamples was performed using a BenchTop Pipettor (Sorenson BioScience; Figure 1D).

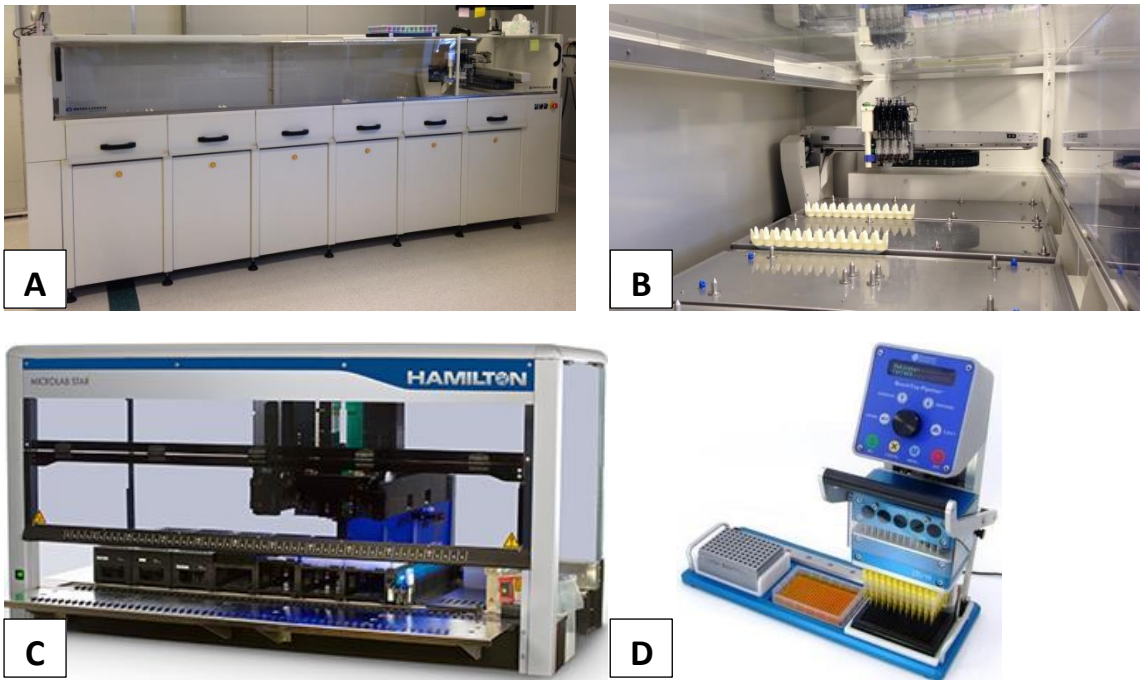

Figure 1. Photographs of the consolidation robot (A, B) used for rearranging vials; Microlab STAR (C) for subsampling milk and BenchTop Pipettor (D) for pooling of milk samples.

2.3 Collection and preparation of serum and faecal samples

Blood samples were collected from 280 animals in 26 herds that were thought to be JD-positive based on ELISA of herd test milk samples. Serum was prepared at LIC Animal Health Lab by centrifuging blood tubes at 3500 rpm for 10 min. Sera were manually subsampled into 96-well storage plates. Faecal samples were also collected from the same animals, using standard rectal sampling and sent to AgResearch (Palmerston North) for culturing.

2.4 ELISA testing – MILK

The IDEXX (previously Pourquier) *Mycobacterium avium ss. paratuberculosis* (MAP) ELISA screening kit was used for testing both individual herd test and bulk tank milk samples. Pooled herd test milk samples were initially tested then individual samples from any reactor pools were tested individually using the same kit to give a result of 'High Positive', 'Positive', 'Suspect' (a group which may have background antibody levels) or 'No antibody detected'. No pooling for Bulk tank milk samples was needed and they were always tested in duplicate by ELISA to minimise the chance of spurious test variation. All test procedures were performed following the manufacturer's instructions. The cut-offs (SP ratios) used for classifying antibody levels were as below for individual herd test milk samples; however, cut-offs for bulk tank milk samples were evaluated as part of this study.

Table 1. ELISA cut-offs (SP ratios) used for diagnosis of individual herd test milk samples

SP Ratio	Diagnosis	Definition
≤20%	No Antibody Detected	No presence of MAP against Antibodies
21-40%	Suspect/Weak positive	Animal needs re-testing
≥40%	Positive	MAP antibodies are present
≥70%	High Positive	High levels of antibodies present

2.5 ELISA testing – SERUM

Prepared serum samples were tested using the same IDEXX ELISA kit and cut-offs used for classifying antibody levels were as per the manufacturer's recommendation for sera samples.

3. Results and Discussion

3.1 ELISA diagnosis for individual animals

Initial herd test screening was carried out using 10 milk samples per pool with a reduced test cut-off to account for the dilution factor occurring with pooling. More recently, LIC's screening strategy was adapted to test pools of eight herd test milk samples. This approach improved sensitivity and resulted in <20% of reactors being missed as a result of the pooling step - mostly weak positives, and a portion of these are likely to be due to contamination or possibly non-specific reactors.

All individuals from any reactor pools were individually tested so every cow received one of four possible test results: A result of 'No antibody detected' indicates no antibody or immunity was detected against MAP in the pooled or individual milk sample, it does not confirm that the cow is uninfected. This is due to the fact that antibody ELISA test will not detect early JD infections; cows may be shedding MAP or advancing towards clinical JD by the following season. Secondly, a 'Suspect' or 'Weak positive' result suggests early-stage JD, or in some cases may be a non-specific reaction or due to carry-over contamination. An animal with this diagnosis is unlikely to be in an advanced stage of the disease and should be retested to assess their JD status. Thirdly a 'Positive' result indicates that the animal is infected with MAP but may be less advanced. Cows may become test positive several years before showing clinical signs due to the nature of this disease. Serum ELISA or faecal PCR can be used to assess their JD status and exclude any false positives due to carry-

over contamination. Finally, a 'High Positive' result indicates that the animal is likely to have well advanced infection or is showing clinical signs of JD. These animals become a major source of contamination of the environment and pooled milk/colostrum.

3.2 Comparison of milk ELISA with serum ELISA and faecal culture

Results for serum ELISA and faecal culture were compared for 280 cows from 26 herds deemed to be 'high positive' (n=233) or 'positive' (n=47) by herd test milk ELISA (Table 2). Ninety eight percent of milk ELISA-high positive cows were also found positive by serum ELISA. However, for milk ELISA-positive animals, only 64% were also positive by serum ELISA, suggesting that some carry-over contamination during herd testing may be causing false positive results. Inclusion of both a 'high positive' and a 'positive' grouping of animals will reduce the impact of contamination on test specificity.

When faeces were cultured, 83% of milk ELISA-high positives and 47% of positives were found to be shedding MAP. While detection of MAP by culture can be difficult due to the intermittent nature of shedding (Mitchell et al., 2015); it is apparent that the 'high positive' group is more likely to have MAP present in the faeces and as such are more high-risk animals for contamination on the farm. By focusing effort on identifying high risk shedders that are spreading infection, diagnostic testing can be a highly effective tool to manage JD risks in the herd.

Table 2. Performance of milk ELISA compared with serum ELISA and faecal culture (JDRC Vet Guide).

	n	Serum ELISA Positive	Faecal Culture Positive
Herd-Test Milk Johne's ELISA PPV:			
'High Positive Cows' only	233	**98%**	**83%**
Remaining 'Positive Cows'	47	64%	47%
All Milk ELISA Positive Cows	280	**92%**	**77%**

3.3 Evaluation of Bulk Tank Milk ELISA as a herd-level risk indicator

Different ELISA cut-offs (SP ratios) were evaluated to optimise BTM ELISA against known JD-positive or JD–negative herds using data from 64 herds (57 classed as JD-positive due to the presence of at least one JD-positive individual). Using a cut-off of SP 0.02, 54 JD-positive herds (95%) were identified as positive by BTM ELISA (Table 3). However, some false positives occurred with this cut-off and three of the seven JD-negative herds were identified as positive by BTM ELISA. When the cut-off was increased to SP 0.05 and 0.1, only 41 (72%) and 23 (40%) of the 57 positive herds were identified as positive by BTM ELISA, respectively. However, the level of false positives decreased as the cut-off increased.

Table 3. BTM ELISA outcomes compared with known herd JD status when three different cut-offs (SP ratios) are used. A total of 64 herds were analysed including 57 defined as JD-positive.

		# HERDS	
BTM ELISA SP ratio	**BTM ELISA result**	**JD-positive**	**JD-negative**
0.02	Not detected	3	4
	Positive	54	3
0.05	Not detected	16	6
	Positive	41	1
0.1	Not detected	34	7
	Positive	23	0

Bulk tank milk ELISA was used to screen herds to identify participants for the 2012-13 JDRC trial; which confirmed the test was acceptable as a positive indicator. However, finding an appropriate cut-off where false positives were minimised while maintaining sensitivity was difficult. To improve specificity, up to 60% of herds with JD-positive animals may be missed. It was also found that identification of a suitable ELISA cut-off was confounded by regional differences in JD prevalence. In an attempt to improve sensitivity, concentrating the antibodies in the milk prior to ELISA testing was evaluated using a commercial kit (Cattletype Milk Prep kit, Qiagen). This approach was unsuccessful as the background levels were too high and, due to the expense and impractical nature of the kit, this approach was not pursued further.

4. Conclusion

Milk ELISA performed well compared with serum ELISA and faecal culture, particularly when including a 'high positive' classification. With pooling, ELISA on herd test milk samples is inexpensive and hassle-free for screening the whole herd for advanced JD cows to cull. Culling will help limit exposure of replacement heifer calves and to minimise clinical disease. The approach of using BTM as a screening tool at whole herd level was unsuccessful due to its low sensitivity and high background, thus alternative screening strategies were investigated.

5. References

JDRC Johne's Disease - laboratory testing - A guide for veterinarians and farmers (dairynz.co.nz)

Mitchell R.M., Schukken Y., Koets A., Weber, M., Bakker D., Stabel J., Whitlock R.H., and Louzoun, Y. 2015. Differences in intermittent and continuous faecal shedding patterns between natural and experimental Mycobacterium avium subspecies paratuberculosis infections in cattle Modelling Johne's disease: From the inside out. Vet Res 46(1): 66