Validation of milk ELISA for detection of Johne's Disease in dairy cows.

This report covers work completed for Milestones 2 & 3 of the Genomics Project:

Milestone 2: Communication plan designed and issued, including provision for a general industry publication.

Milestone 3: Complete validation of milk ELISA for screening.

Executive Summary

The ultimate objective of this project is to identify genetic markers for susceptibility and resistance to Johne's disease in dairy cows. Over the last 7 months, the LIC project team has completed the first 3 milestones of this Objective in a timely fashion and within budget. A robust validation of the use of the ELISA test and proposed sampling regime has been conducted.

An operational plan is now in place to commence a large screening programme within the national dairy herd during Year 2 of the programme.

Key Messages

- Milestone Aims: to establish a communication plan and to complete the validation of the ELISA test and sampling regime.

- An LIC communication plan has been written and circulated to JDRC.

- The ELISA test and sampling regime has been robustly tested and provides a cost-effective method to commence a large scale screening for Johne's disease in the national dairy herd.

- It is proposed to vat test approx 4000 herds to target 400 herds for pooling and sampling of individual cows. It is hoped to source approximately 1000 affected animals.

Milestone 2: Communication Plan

An LIC communication plan has developed and circulated to JDRC.

- A farmer update on results from the pilot trial and industry article are currently being prepared.
- Hinrich Voges is to present .2 abstracts at the 10th International Colloquium of ParaTb, Minneapolis on 1) analysis of culling data and 2) use of a bulk milk ELISA as a herd screening tool.
- Farmer updates/industry articles are scheduled for July & November 2009, July 2010, and July 2011.

Milestone 3: Completed validation of milk ELISA for screening

The validation of the milk ELISA as a screening tool has been completed. All selected animals have been sampled and farmers have been informed of their results. As discussed in the Milestone 1 report, the proposed sampling regime is a robust and cost-effective way to screen a large number of dairy cows to sample affected animals and this has been confirmed by the completion of testing the selected herds.

In total, to select 56 herds to sample affected cows from:

- 2,081 pools were tested
- 3,351 cows were tested individually
- 18,922 samples were screened

The sero-prevalence of Johne's disease (milk ELISA positive) in the 56 herds selected on the basis of presumed Johne's Disease-risk ie vat test results (Table 1) and Johne's Disease culling history are shown in the following tables. Herds across the spectrum of Johne's Disease-risk were targeted, however a there was a strong bias to high-risk herds. In agreement with results reported in the Milestone 1 report, there is a low prevalence of Johne's Disease in the national dairy herd as shown by age (Table 2) and breed (Table 3). As expected, there was a higher prevalence of Johne's Disease shown in older animals.

Table 1 Vat test results

Vat test result	Herds	Individual samples	Herd Test sero-prevalence (%)
<0.05	22	6 544	0.74
0.05 - <0.1	19	6 815	2.01
>=0.1	23	5 563	5.29
All herds	64	18 922	2.75

The trial also indicated that herds with vat test results S/P>=0.1 are likely to make up less than 1% of the national herd. We will still fine-tune the process, but at this stage we expect to target herds with vat test result S/P>0.4 for herd test screening. The vat testing process for the pilot trial and screening is confidential and is kindly supported by Fonterra and Westland.

Table 2. Sero-prevalence by age.

Age	Year of birth	Number of cows	% Positive samples
2	2006	1983	1.01
3	2005	3939	1.60
4	2004	3127	2.94
5	2003	2477	2.30
6	2002	2092	2.87
7	2001	1783	3.42
8	2000	1417	3.60
9	1999	822	3.28
10+	1998 -	1282	2.26
Overall		18922	2.43

Predominantly 3 year and older cows were screened. However, a subset of 2-year-olds (<50%) was screened with a strong bias towards herds with high vat test results ie greater within-herd Johne's Disease prevalence.

Table 3 Sero-prevalence by breed.

Breed	Number of cows	% Positive samples
Jersey (> 13/16)	11033	3.1
HF X J	2295	2.7
Holstein Friesian (> 13/16)	4972	0.9
Misc	622	2.1
Overall	18922	2.75

HF x J have defined parentage to 16/16 Holstein Friesian and/or Jersey, while Misc may have other breeds (A > 13/16, Cross Breed, (blank), Brown Swiss > 13/16, Beef Breed > 13/16, SH > 13/16) in them or not-defined.

The farmer-owners of the 56 herds selected were contacted, and 50 agreed to participate in the pilot trial, which was a very positive result. Due to sample collection being late in the season, cows were sampled on 47 farms as some cows had already been culled or dried off. In total, 286 cows were individually sampled for blood, milk and faeces by AsureQuality technicians. On receipt at the lab, faecal samples were sub-sampled for sample storage for PCR, and sent to Wallaceville for culture. 247 blood samples from affected animals were selected for DNA storage and 31 were rejected. This was due to cows testing positive on herd test milk samples but either showed low positive reactivity or were negative on serum confirmation testing. Most of these animals are likely to be in the early phase of disease.

Figures 1 and 2 show the relationships between the herd test, individual milk and blood test results. Figure 1 shows that with the assay cut-off levels that were tested in the pilot trial, a high proportion of animals that tested positive on their milk sample tested positive on serum.

Figure 1 Relationship between individual milk ELISA results and confirmation blood (serum) results.

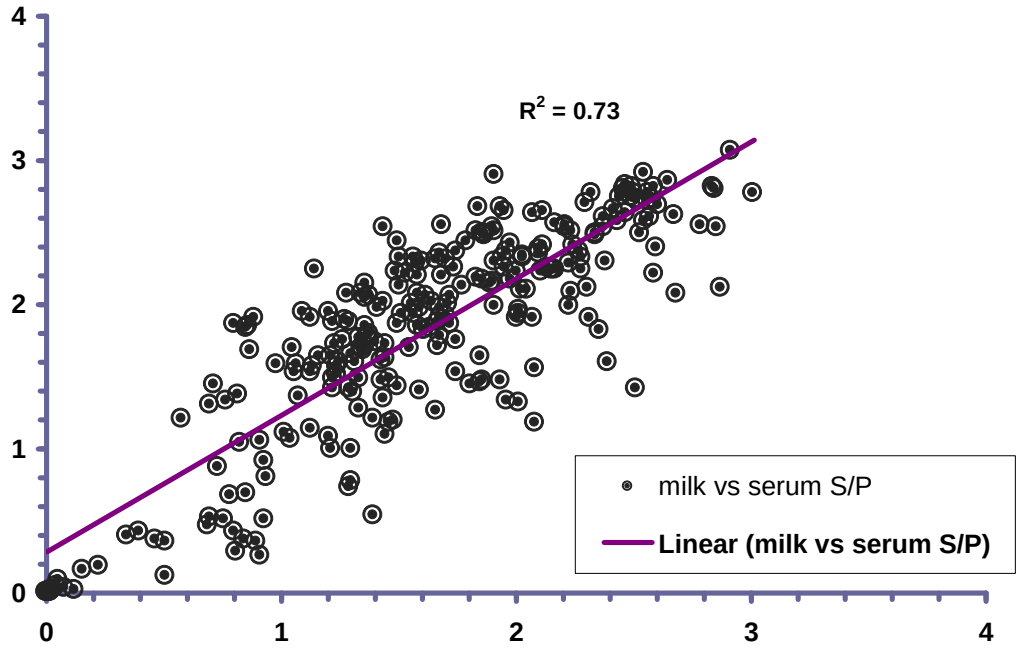
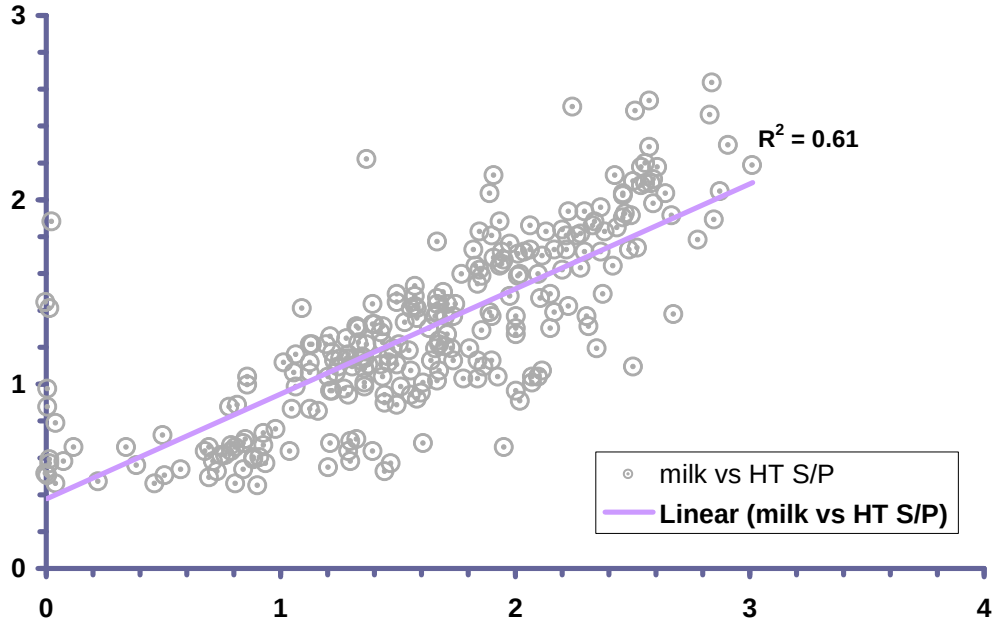


Figure 2 shows that high proportion of animals that tested positive on herd test samples tested positive on the individual milk sample.

Figure 2 Relationship between milk herd test ELISA results and individual milk samples.



The LIC ELISA validation trial was also successful in terms of supporting the collaborative aims of the JDRC experimental programme as it was able to provide faecal samples for Milestone 1 Microbial Quantification to culture different isolates, and information to help source cows for Milestone 3.1 Mucosal Immune Response.

In conclusion, LIC is very confident that this sampling regime can be used to screen a large number of dairy cows in a cost-effective manner. Next season it is proposed to vat test approx 4000 herds (agreement is in place from the dairy companies to supply samples) to target 400 herds for pooling and sampling of individual cows. It is hoped to source approximately 1000 affected animals. The proposed timeline is:

- August – September 2009 herd identification.
- October – December 2009 Vat sample screening.
- January – April 2010 Herd test selected herds.
- February – April 2010 Sample identified cows.
- May 2010 – Reporting completed.

Finally, a brief update on the scoping of a PCR method for detection of MAP in milk and faecal samples. A PCR method is being trialled on faecal and milk samples collected during the pilot trial to potentially provide extra phenotypic information. The added value of this will depend on the accuracy of the test and the cost of samples. This will be further examined.