

Milestone 1. Interim validation of milk ELISA for screening.

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

This report covers work completed for Milestone 1: Interim validation of milk ELISA for screening dairy cows for Johne's Disease.

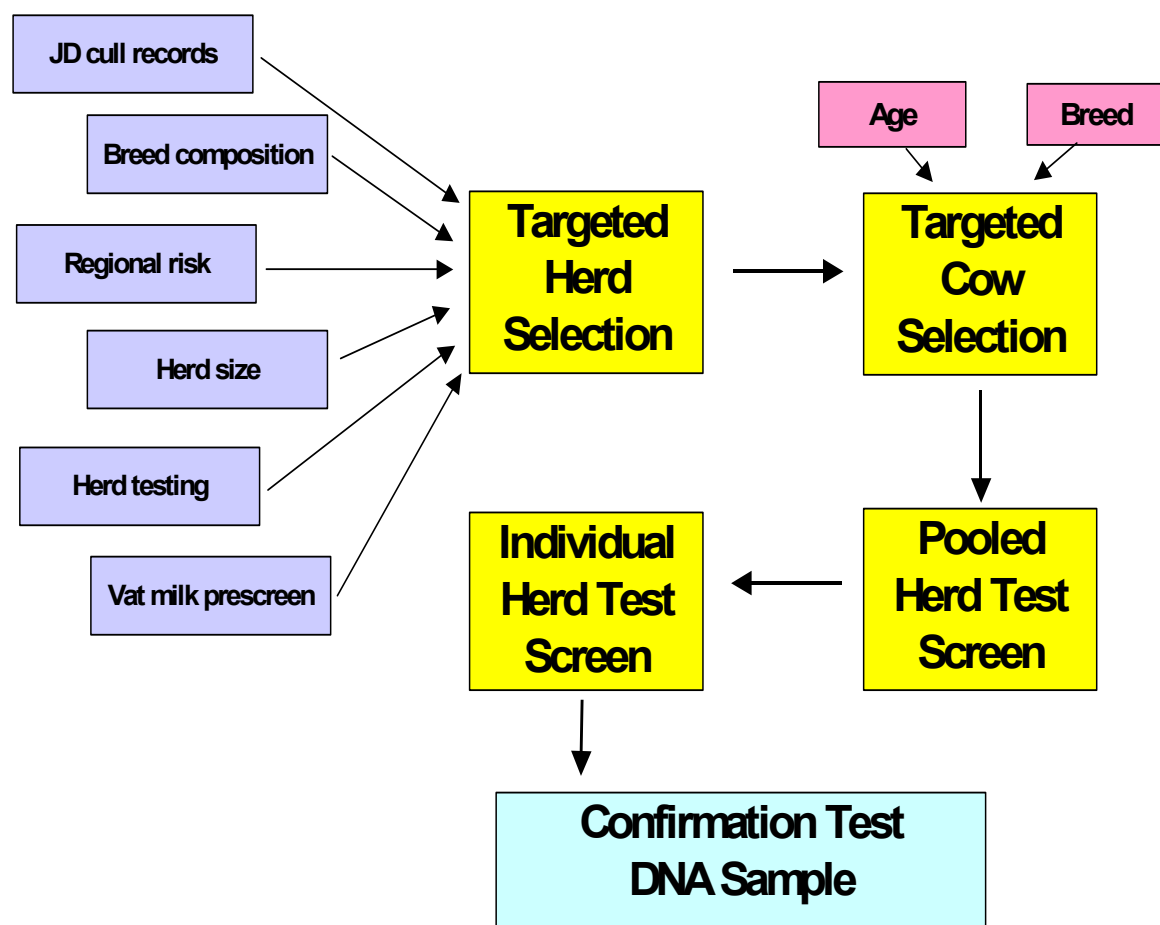
### Summary

- The aim of this part of the project was to test the process of using milk samples to allow targeted sampling of dairy cows to collect and store DNA samples from Johne's Disease case animals.
- Milk bulk (vat) test was found to be a very useful tool for pre-screening to allow targeting of herds with affected cows.
- Pool – individual testing of herd test samples shows a strong correlation between the vat test and proportion of Johne's Disease positive cows in the herd.
- Pool test approach has resulted in a 75% test reduction.
- Only 2% of milk samples tested have tested positive despite inclusion of high risk Johne's Disease herds in the trial. At this stage, this suggests the optimal protocol will require vat screening of most or all herd-testing herds.

### **1. Identification of infected animals**

Use of milk processing companies vat samples and herd test samples has been utilised to indirectly sample a large number of dairy cows. Figure 1 describes the factors considered in the method of identification of animals for DNA sampling. The confirmation test will be done a blood sample that will also be used for DNA extraction for subsequent storage and analysis.

Figure 1. Proposed method of identification of JD-case animals.



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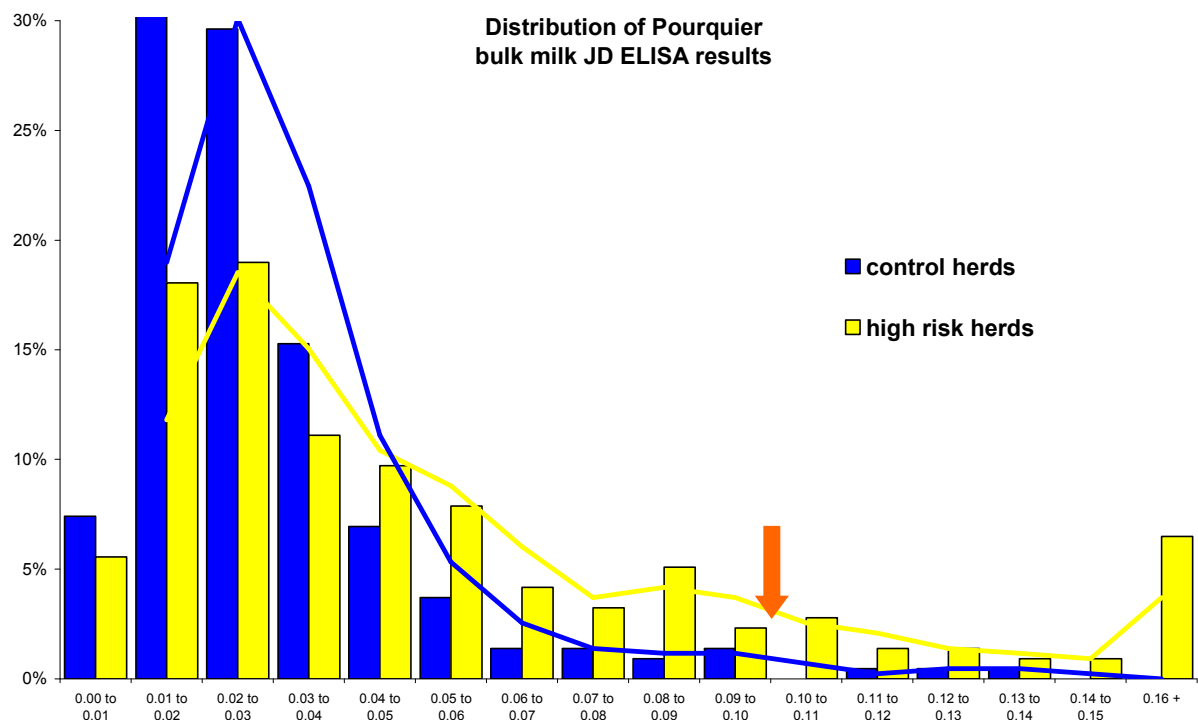
## 2. **Bulk (vat) milk pre-screen pilot study**

A pilot study to determine the validity of using the farm vat sample as a screening tool was conducted during January-February 09 using 400 herds from

- a) low prevalence regions and
- b) high risk regions and herds that cull for Johne's Disease.

Results are shown graphically in Figure 2. The graph shows a distinct right shift of case vs control herds, suggesting that the bulk milk ELISA may be an efficient screening tool to target high(er) Johne's Disease prevalence herds. To test the validity of the bulk (vat) milk test and understand its relationship with the proportion of Johne's Disease reactor cows within the herd, we selected 60 herds from across the range of test results, with a bias towards the top end of the graph below. The herd test samples of cows greater than 2 years old are being tested after pooling (10:1) with an individual retest of pools showing potential reactors.

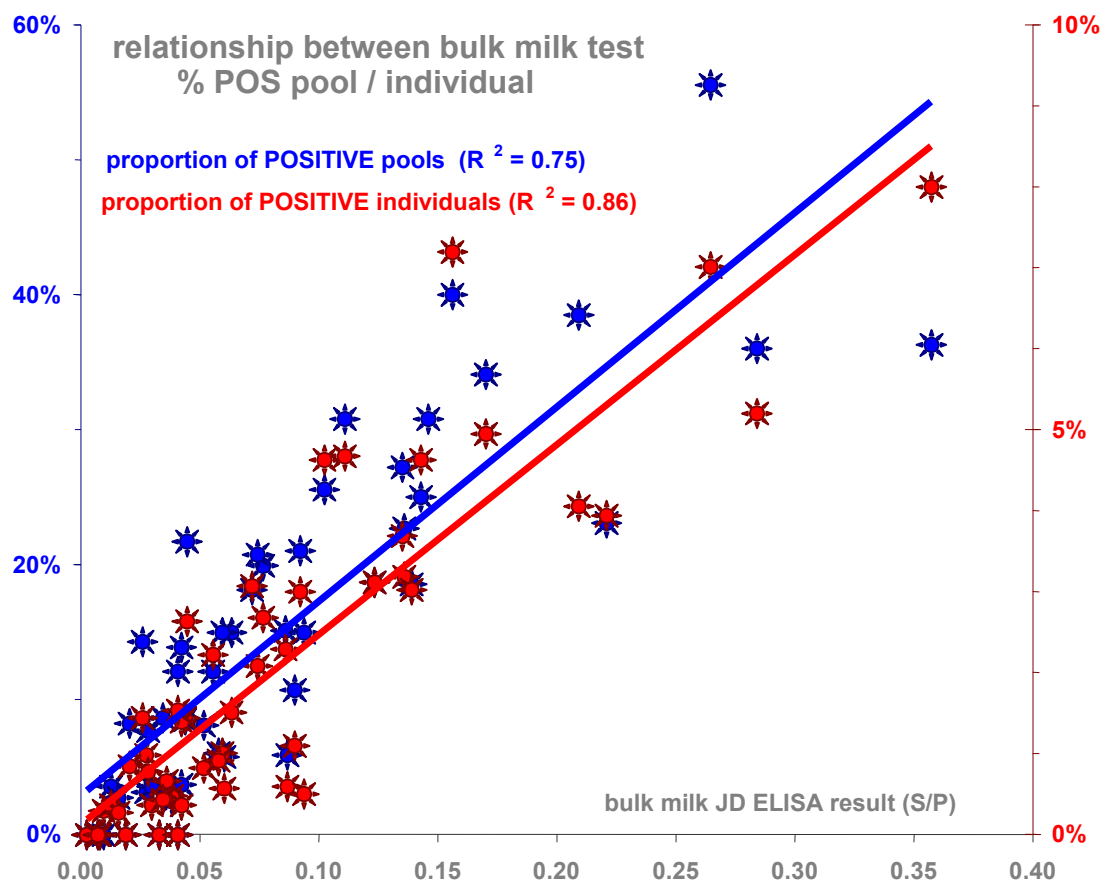
Figure 2. Vat milk pre-screen pilot study results.



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To date, pool and individual testing of herd test samples from 40 herds has been completed. Figure 3 demonstrates there is a strong correlation between the bulk (vat) test result (x-axis) and the proportion of Johne's Disease positive cows in the herd (red dots). So the vat test will be a powerful tool to help target herds with higher Johne's Disease risks for the pool - individual cow testing process to identify affected cows for the genomic study.

Figure 3. Herd bulk (vat) test results versus individual cow screening.

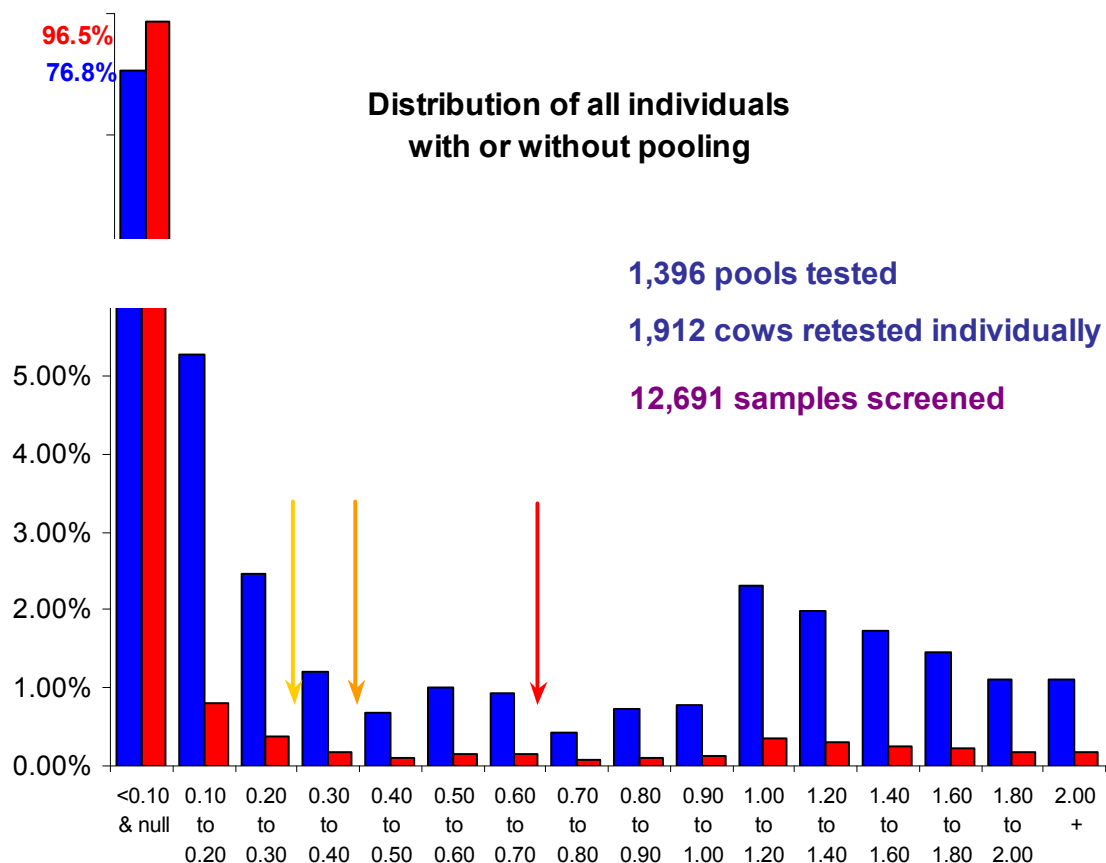


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### 3. Pool - individual cow screening

So far, over 12,500 cows from 40 herds have been screened using the pool and then individual test of cows from potential reactor pools approach with herd test samples. With almost 1400 pools and 2000 individual samples tested, the pooling strategy has resulted in a 75% test reduction.

Figure 4. Distribution of pool – individual ELISA test results (blue) with hypothetical distribution of no pooling used (red). shows the distribution of pools tested.



However, only 2% of the milk samples have tested positive despite the inclusion of high Johne's Disease risk herds in the trial. This result reaffirms that the expected Johne's case rate in the dairy cow population is low. Targeting of herds with higher Johne's Disease risk therefore has to be a crucial component of our approach. Current data suggests that the optimum protocol will require vat screening of all or most herd-testing herds. Once the validation trial data is complete, the testing programme will be fine-tuned.