JDRC Report on Validation Study

Executive Summary

- A validation study was undertaken to confirm the higher than expected prediction accuracies of the model developed in the initial trial
- The prediction accuracies of the model were as expected (AUC of ~75%) for cows born prior to 2009
- The prediction accuracies for cows born in or after 2009 were lower than expected (~55%) suggesting a requirement for on-going additional genotypes to maintain predictive performance of the model
- Including validation samples in the original model made only a small improvement (2%) to the accuracy of the predictions.
- The on-going cost of genotyping may outweigh the value of the predicted JDS BVs.

Introduction

The original set of animals and samples gathered as part of the project were used to develop a Johne's Disease Susceptibility BV (JDS BV) as a prediction of the susceptibility of an animal to Johne's Disease (JD) based on its genotype. Estimates of the accuracy of the prediction obtained via cross-validation were very high with an average area under the curve (AUC) of over 90%. However this level of accuracy was significantly higher than would be expected from a trait with an estimated heritability of 0.22 (Back and Lopez-Villalobos, 2012).

The Science Review Board suggested additional work be undertaken to examine whether the higher than expected accuracy was a result of close relationships between animals in different folds of the cross-validation. Reducing the relationships between cross-validation folds did not significantly change the estimates of the prediction accuracy.

Given the high accuracies were still unexplained it was determined prudent to undertake a validation study to confirm the prediction accuracy. The study reported here aimed to validate the accuracy of the predictive tool in a separate cohort of animals.

Methods

The genomic prediction of JD susceptibility for cows identified as Johne's disease positive (JD+) and their test-negative herd-mates (JD-) were compared to each cow's JD status to determine the accuracy of the predictive test for susceptibility to JD.

Johne's disease diagnosis

The trial used a matched case/control design rather than the Wellcome Trust approach (Wellcome Trust Case Control Consortium, 2007) used in the initial trial to improve the power of the trial while testing fewer animals.
Diagnostic testing of milk and blood samples employed an enzyme-linked immunosorbent assay (ELISA) marketed as the IDEXX Paratuberculosis Screening Ab Test (www.idexx.com).

Potential candidate cows where identified from 24 herds that screened their routine herd test milk samples for JD in 2015. Each candidate JD+ cow was matched to a test-negative herd-mate (JD-)

that was born in the same year and birth herd and had the same breed composition to try and ensure that both cows had similar exposure and a similar time to incubate the disease.

Subsequently, blood plasma and faecal samples were collected from candidate cows to confirm the ELISA positive status. JD+ cows were selected using an increased test cut off (0.75 vs 0.40) to target cows with more advanced JD, while matched JD- cows were selected from ELISA negative herd mates with a very low results (<0.10 vs <0.20).

Faecal samples from candidate cows were tested by quantitative PCR. JD- cows had to be either PCR negative or excreting less than 1/100 as much as the matched JD+ cow.

Only matched pairs of candidates passing all milk, plasma and faecal tests were eligible for the trial.

Genotypes

DNA for genotyping was extracted from tissue punches, collected at the same time as the blood and faeces used to confirm JD diagnosis. Genotyping was performed using the GeneSeek Genomic Profiler HD-150K (GGP HD-150K) and resulted in 111 matched JD+, JD- pairs and 3 additional un-matched JD+ samples with sample rates of 95% or greater.

All genotypes were imputed to the SNP set utilised to predict JDS BVs using Beagle v4 (Browning and Browning (2009)).

Analysis

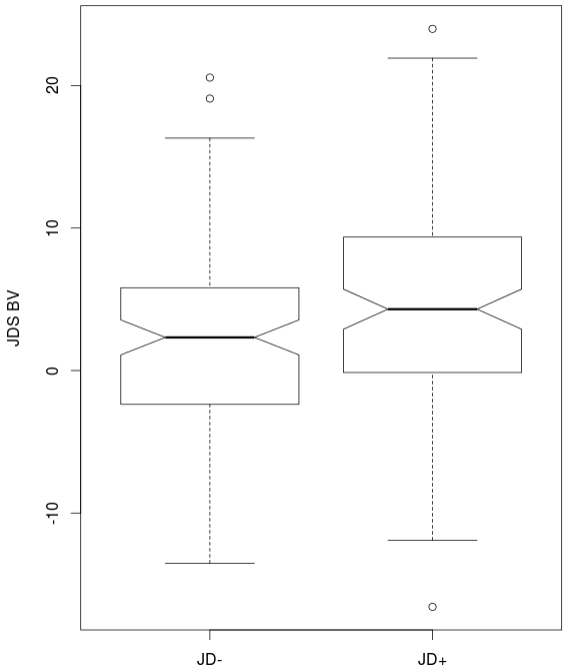
The predictive genomic tool developed from the initial trial (Sherlock *et. al.* 2014) was applied to the 225 validation genotypes to predict their JD status.

Ten-fold cross-validation of the validation samples was also undertaken as described by Sherlock *et. al.* (2014) to examine whether adding newer data would improve predictions. Each pair of matched validation samples was randomly allocated to one of 10 folds. The data from the initial trial along with 9 folds of validation data were used to predict the 10th fold of validation data, the folds were rotated so that all validation animals were predicted.

Results and Discussion

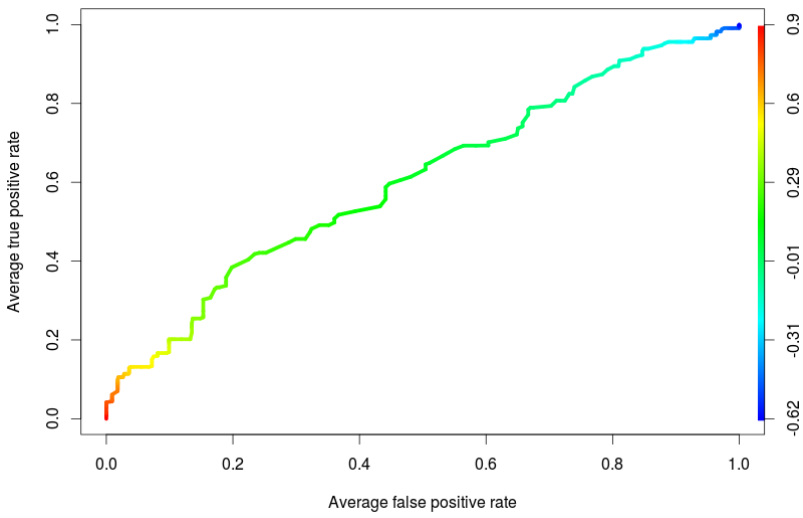
Figure 1 show that the mean predicted susceptibility of the JD+ cows was slightly higher than for the JD- cows but not significantly so.

Figure 1: Comparison of Johne's Disease Susceptibility BV (JDS BV) for JD+ and JD- validation cows predicted using the model developed in the initial trial.



This results in a Receiver Operating Characteristics (ROC) curve (Figure 2) with an area under the curve (AUC) of 61%. The AUC represents the probability that a randomly selected JD+ has a higher predicted susceptibility than a randomly selected JD- cow. This result is much lower than the ~90% AUC obtained in the cross-validation results from the trial used to develop the predictive test, confirming that the original results over-estimated the accuracy of the predictive test. At a level of 61% the accuracy of the test would be classified as poor but is comparable with results from a similar analysis in US Holstein cattle by Alpay *et. al.* (2014) who obtained an average AUC across 5-folds of 55%.

Figure 2: Receiver operating characteristic (ROC) curve for validation cows.



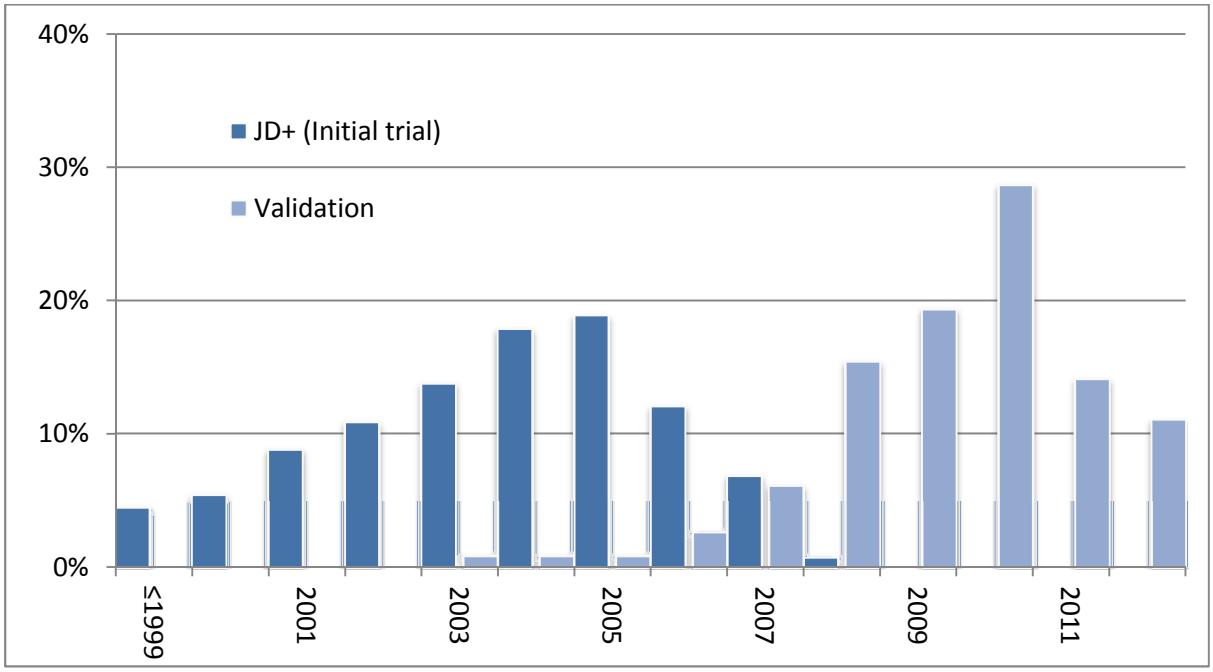
Further investigation of the results suggested that predictions were more accurate for older cows in the validation, than for younger ones, in particular the JD status of cows born before 2009 was predicted more accurately than that of cows born in 2009 and later with AUCs of 75% and 55% respectively (see Table 1).

Table 1: Area under the Receiver Operating Characteristic curve (AUC) by year of birth (YOB) of validation cows.

Year of Birth	n	AUC (%)
≤2006	10	64.0
2007	14	83.7
2008	35	74.5
2009	44	56.0
2010	65	54.4
2011	32	55.9
2012	25	55.8

Figure 3 shows that while the initial trial included some JD+ animals born as late as 2008, there were no JD+ animals born later than that. This coincided with the drop in prediction accuracy of the validation animals born in 2009 and later.

Figure 3: Histogram of year of birth for JD+ cows from the initial trial and for validation cows.



Better predictive results are generally obtained when the animals used to develop the prediction are more closely related to the animals being predicted. In this study there was an average coancestry (relatedness) of 1.8% between the JD+ animals in the initial trial and the validation animals born before 2009 and a very similar but slightly higher coancestry of 2.0% between the JD+ animals in the initial trial and the validation animals born in or after 2009. This suggests that differences in relatedness of the training and test populations were not responsible for the drop in prediction accuracy.

Prediction accuracy might also drop if the genotypes that were susceptible to JD prior to 2009 are not the same as those that are susceptible in later years – perhaps due to changes in the strain of the bacterium or the mode of transmission. If this were the case, then augmenting the original (pre-2009) data with information on genotypes and JD status from later years should improve prediction accuracy. Figure 4 shows the JDS BV predictions generated by adding 10-fold cross-validation data from the validation set of animals to the original training data. The figure shows that the difference between the mean predicted susceptibility of the JD+ cows and that of the JD- cows was a little bigger than for analysis that only used the original training data.

Figure 4: Comparison of Johne's Disease Susceptibility BV (JDS BV) for JD+ and JD- validation cows predicted using the model developed in the initial trial.

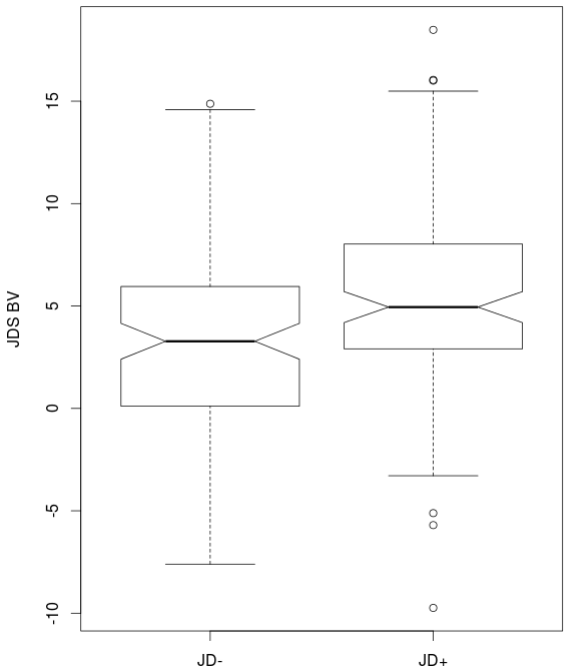


Figure 5 shows the ROC curves obtained from 10-fold cross-validation of the validation samples (all samples from the initial trial were included in each of the 10 training sets). There was a large amount of variability in the prediction accuracy of the individual folds which was to be expected given the small number of cows in each fold. The shape of the average curve improved a little and the AUC increased from 60.6% to 63.2% (Table 2). There was some improvement in the prediction accuracy of the cows born in 2009 and later, but not to the levels of cows born prior to 2009. Increasing numbers of younger cows genotyped may improve prediction accuracy further.

Figure 5: Receiver Operating Characteristic curves for 10-fold cross-validation analysis showing the ability of JDS BV to predict the JD status of validation cows.

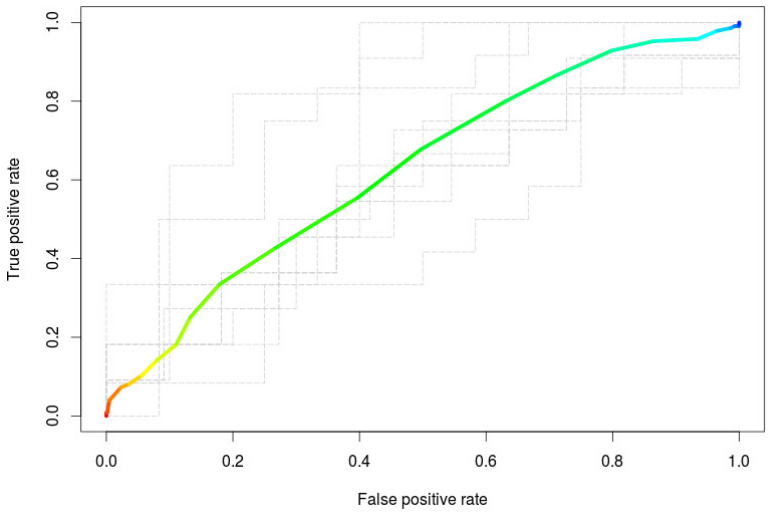


Table 2: Area under the Receiver Operating Characteristic curve (AUC) for 10-fold cross-validation of validation cows

	<2009	≥2009	All
Original test	74.5%	55.4%	60.6%
Augmented test	76.6%	57.6%	63.2%

Conclusion

The predictive test had poor accuracies for validation cows born in years that were not represented in the population used to develop the test. There was some evidence that updating the training population used to develop the test may help maintain the prediction performance of the test over time, however improvements were small and suggest a large investment in on-going genotyping may be required to provide an accurate test.

The study suggests that the test will be not be as useful for identifying young stock that are less susceptible to JD because appropriate training data will not be available until they are older. The JDS BV may remain a valuable tool for selecting mature bulls to mate with a herd to decrease its susceptibility to JD. However this is dependent on the cost of on-going genotyping relative to the value of the JDS BV in decreasing susceptibility to JD.

References

Alpay F., Zare Y., Kamalludin M.H., Huang X., Shi X., et al. (2014) Genome-Wide Association Study of Susceptibility to Infection by Mycobacterium avium Subspecies paratuberculosis in Holstein Cattle. PLoS ONE 9(12): e111704. doi:10.1371/journal.pone.0111704

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