3.4.1 Milestone 4.1.1 Archive of Ruminant DNA – Cervine

Description	The objective of this milestone is to build up an archive of DNA samples. The occurrence of Johne's Disease, especially in cattle is sporadic and unpredictable. It is a straight forward exercise to bleed and prepare DNA from 2000 ruminant animals. The long time frame is due to the difficulties envisaged in identifying suitable cattle. Deer, where the disease is more prevalent, and large numbers of clinical Johnes can be found within a single herd, should give large enough numbers of animals within the first year. By 2012 the aim is to have the following DNA's archived from each species: 2000 Dairy Cattle DNA's; **2000 Deer DNA's.**
Alignment with JDRC Strategy	Tool: Database to support development of gene markers for identification of Johne's disease resistant animals
Status	95% Complete,

2011 Science Report

Final Report

Archive of cervine DNA
Milestone # 4.1.1

Allan Crawford
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Executive Summary

The object of this study was to prepare a DNA archive from deer that had been measured using the DRL Paralisa™ test developed by Frank Griffin and colleagues. The archive now contains DNA samples comprising 970 deer with high antibody levels against the causative agent of Johne's disease *Mycobacterium avium spp paratuberclosis* (MAP). Along with these samples we have collected a DNA sample from a matched herd mate that shows little or no evidence of any immune response. As the goal is for 1000 samples from each of the two groups we will have completed the three year project well before the deadline of 30th June 2011.

Introduction

Currently the Paralisa™ test for MAP antibodies is the only method available to deer farmers to help control Johne's disease (JD) in their herds. The test has been used to identify heavily infected animals for culling and has reduced the incidence of Johne's disease on farms using this test and cull strategy. The Paralisa™ test is used after animals have been exposed to the disease. A predictive DNA test used prior to exposure would add a further very useful tool for the industry in its attempts to control this worldwide pathogen of ruminants.

The rapid development over the last five years of both DNA sequencing and its subsequent analysis has enabled large scale genome wide association studies to be undertaken. A common design for these studies is to compare the DNA of two populations that differ in a particular trait. The outcome from these studies is knowledge of what regions are contributing to genetic control of the trait. Based on that knowledge a DNA test can then be developed which will predict the trait in the animal tested.

The goal of the milestone was to establish a DNA archive from deer that could be used for a Genome-wide Association study. Obtaining DNA from large numbers of animals that have been accurately measured for a trait, especially traits involving a disease which farmers would like to eradicate is often difficult. Fortunately we were able to make use of the samples sent to the DRL for Paralisa™ testing. We were still however dependent on the rate of submission to the laboratory. Fortunately the throughput of the lab has remained high over the last 3 years and we will be able to reach our goal.

Methods

Deer Phenotypes:
In the first year we purified DNA from all deer that provided a positive Paralisa™ test along with a herd matched control were collected. Following new advice from the DRL we further restricted our collection in the 2nd and 3rd years to only those animals whose Paralisa™ absorbance reading was greater than 100 for either the Johnin or PPA antigens. The reason for the change of policy was the finding that deer with Paralisa™ titres >100 are likely to be significant shedders of MAP bacteria (so called "super shedders") and develop clinical symptoms.

DNA Purification:
Blood for the Paralisa™ test is sent in vaccutainers. All blood provided in heparin treated vaccutainers that had the appropriate phenotype was obtained from Simon Liggett at DRL. The DNA was purified using the method of (Montgomery and Sise 1990). This method provides very pure DNA that has been shown to be suitable for all forms of DNA analysis including high-throughput sequencing and Illumina SNP genotyping. After purification DNA concentration was measured and any samples at less than 2 ng DNA / ul were discarded.

Data and sample storage:
All DNA samples are stored at -20^{o}C in screw capped plastic tubes labeled and bar-coded with their sample number. All data relating to that sample including: sample number, farm of origin*, animal tag number, DRL accession number and measurement date, Johnin, PPA and EVA titres, DNA concentration, are stored in a relational database that is regularly backed up.
* (two farms wished that their use of the Paralisa™ service to remain confidential so these 72 samples have an unknown farm of origin)

Results:

A total of 2026 DNA samples from 36 properties have now been purified and archived. Only 38 of these samples have between 2 and 10 ng / ul which may require the DNA to be concentrated before use. The average DNA concentration of the archive is 226 ng / ul. 970 samples have positive Paralisa results with 242 being between 50 and 100 Abs. units and 728 being > 100 Abs. units in either PPA or Johnin titre. The remaining 1056 samples are herd matched negative controls. Table 1 contains a list of the properties and the number of samples we have so far obtained from each.

Table 1: Deer farms or owners that have contributed to the DNA archive at March 2011

Property or owner	# of DNA samples
Landcorp, Stuart	524
Landcorp ,Rangitaiki	222
Holden	208
Landcorp, Freestone Wapiti	164

Landcorp, Butlers	134
Black Forest Park	124
Aitken	90
Peel Forest	72
Unknown #1	54
Johnston	52
Landcorp, Cape Foulwind	43
Hakataramea	32
Minaret Station	30
Invermay	28
Landcorp, Raft Creek	28
Liggett	22
Jardine	20
Allan	19
Unknown #2	18
Love	16
Mendip	16
Landcorp, Mararoa	14
Mt Sommers	14
Remarkables Park	14
Garden	10
Telford Deer	10
Totara Hills	10
Beechwood Hills	8
Matthews	8
Fleet	6
Proudfoot	6
McDonald	4
Burdon	2
Edendale Station	2
Glen Isla Elk	2
Landcorp, Mt Hamilton	2

Conclusion:

A DNA archive established for Genome-wide association studies for Johne's disease resistance in deer is on track to be completed by 30th June 2011.

References:

Montgomery G.W. & Sise J.A. (1990) Extraction of DNA from sheep white blood cells. *New Zealand Journal of Agricultural Research* **33**, 437-41

Acknowledgements

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