2008–2016

RESEARCH AND DEVELOPMENT RECORD June 2016

JOHNE'S DISEASE
RESEARCH CONSORTIUM

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Glossary

AgR	AgResearch Limited
B+LNZ	Beef + Lamb New Zealand Limited
DCANZ	Dairy Companies Association of New Zealand
DNZ	DairyNZ Limited
DINZ	Deer Industry New Zealand
ELISA	Enzyme Linked Immunosorbent Assay
In vitro	Literally "within the glass", refers to experimental systems in containers in contrast to the natural system
In vivo	Literally, "within the living", refers to experiments using a whole, living organism
JAG	Johne's Advisory Group
JDRC	Johne's Disease Research Consortium
JDRL	Johne's Disease Research Limited
JML	Johne's Management Limited
IP	Intellectual property
LIC	Livestock Improvement Corporation
MAP	*Mycobacterium avium paratuberculosis* —the bacterium that causes Johne's disease
MIA	Meat Industry Association
MBIE	Ministry of Business, Innovation and Employment. www.mbie.govt.nz (formerly Ministry of Science and Innovation, MSI)
NZ	New Zealand
OJD	Ovine Johne's disease
Paratuberculosis	Another name for Johne's disease (PTB)
PCR	Polymerase chain reaction
PGP	Primary Growth Partnerships
PTB	Paratuberculosis
R&D	Research and development
SAG	Science Advisory Group
SNP	Single nucleotide polymorphism, genetic variations in the DNA of individuals that can be used to differentiate them and to identify variations in regions of their genomes
UJV	Unincorporated Joint Venture

Foreword by the Chairman

The idea of bringing together a single group to manage research into Johne's disease (JD) in New Zealand was first conceived in 2006. While there was research being undertaken and industries investing in solutions, there was no coordinated plan for an issue which had the potential for wide cross sector impact. The core of the problem was that the industry did not understand the true impact of JD in New Zealand livestock or the best way to manage the disease in a New Zealand setting. The establishment of the Johne's Disease Research Consortium by industry, with support funding from Government, has been a major step in bringing New Zealand Inc's knowledge and ability on how to manage Johne's disease into a secure position for the future.

2016 sees us much better equipped to manage Johne's disease in New Zealand. We have an enhanced understanding of the disease itself, its impact and cost to farmers and how this varies from sector to sector and most importantly, through the efforts of the JDRC and other groups in New Zealand, we now have the tools for farmers that should allow for the practical management of JD on affected properties.

Johne's is a difficult disease to study. On behalf of the Board I would like to commend the many researchers who have worked on this project. Their skill, time and effort has resulted in many new discoveries about the bacteria itself, disease mechanisms, the genotypes of affected animals and the farming techniques required to reduce Johne's impact. The work could not have been done without considerable dedication to this worthy task, as testified by this document. Many of New Zealand's internationally recognised science leaders in JD and *Mycobacterium avium paratuberculosis* (MAP) have or are soon to retire. We trust that you will have long and happy retirements but also that you will take every opportunity to train up the next generation of JD experts. They will continue to be needed.

While there is still more to learn about JD, we are confident that the big questions for New Zealand have been answered sufficiently well that continued investment by the Consortium is not required. We leave behind the "Johne's Advisory Group", a cross sector group of experts to watch over research in JD and the tools developed to ensure industry information remains relevant. The Consortium itself will close on the 30 June 2016 satisfied that the mandate to deliver cost effective tools to farmers for the management of JD in New Zealand has been achieved.

It has been my pleasure to chair the Consortium Board since 2011. The JDRC Board draws its members from each of our participating organisations, each of whom has an active interest in science, on-farm issues and providing practical tools for our ultimate shareholders to implement at a farm level.

I would like to acknowledge Dr. Andrew MacPherson, who chaired the Consortium for the first three years. Also the many directors and advisors who gave considerable time and effort to this cause, but in particular special thanks must go to Dr. Lindsay Burton, Dr. Eric Hillerton, Dr. Mandy Bell and Dr. Steve Harcourt.

And finally our Consortium Manager, Kaylene Larking, has been not only diligent and committed, but amazingly adept at bringing often countervailing views and motivations together to work for a common purpose. Thank you Kaylene.

We trust that the Consortium's findings will continue to be of considerable value to all involved in the New Zealand livestock industry.

Graeme Milne
Chairman
Johne's Disease Research Consortium

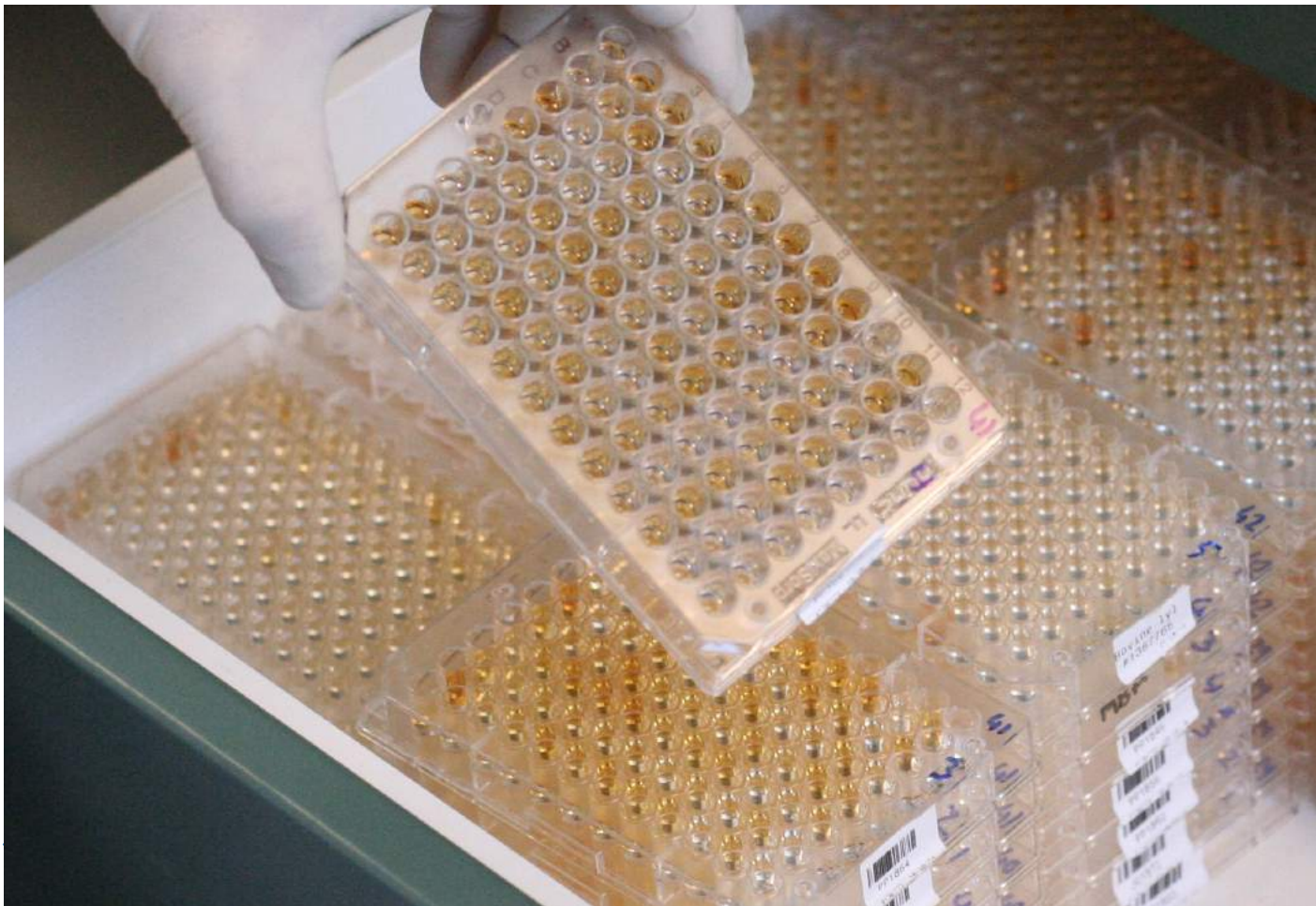
Introduction

The Johne's Disease Research Consortium Research and Development Record has been assembled to chronicle the work of the Consortium and the team of researchers who have worked with industry to develop practical tools for the management of Johne's disease (JD) in New Zealand over the last eight years.

The aim of the JDRC research and development (R&D) programme was to deliver practical tools for farmers to manage JD and that has been largely achieved. The outcomes delivered to farmers from our programme are underpinned by fundamental scientific information about *Mycobacterium avium paratuberculosis* (MAP) and how it impacts livestock, but the solutions recommended are very simple in implementation. Our work indicates that JD is a low level threat to most New Zealand farmers but for a small set of producers this disease is causing significant loss. Being responsive to the signs of disease in a flock or herd and implementing the best practice tools make it possible to bring the disease under control for most farmers.

This document summarises the work of the Johne's Disease Research Consortium (JDRC). It outlines the intent of the R&D programme, what was found and how those findings have been integrated into management programmes designed for the individual livestock sectors. The document also describes the work of the Consortium to integrate JDRC findings and other international and local developments into the creation of resources for New Zealand farmers.

Kaylene Larking
Consortium Manager
Johne's Disease Research Consortium (JDRC)



The Consortium

The Johne's Disease Research Consortium was established in 2008 as a joint venture between industry, government and the science community to coordinate Johne's disease research in New Zealand. The Consortium closes in June 2016 after eight years of operation having substantially completed its objective to support the development of cost effective tools for the management of JD on New Zealand farms.

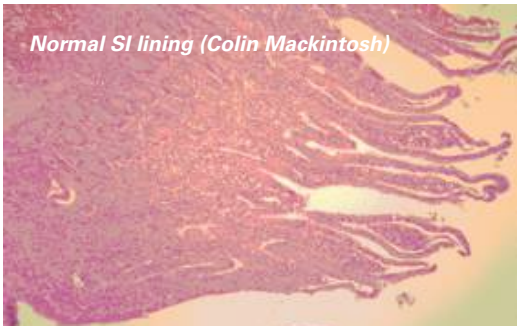
During its term the JDRC invested $10.4 million in JD research behind the farm gate, looking at diagnostics, genetics, epidemiology and fundamental aspects of JD, to develop and refine practical and cost-effective tools for sheep, cattle and deer. The JDRC played a key role in JD research in New Zealand; investing in the local research community, supporting the task of understanding the impact of JD for the New Zealand context and in ensuring that there are tools in place to control the disease for all farmers.

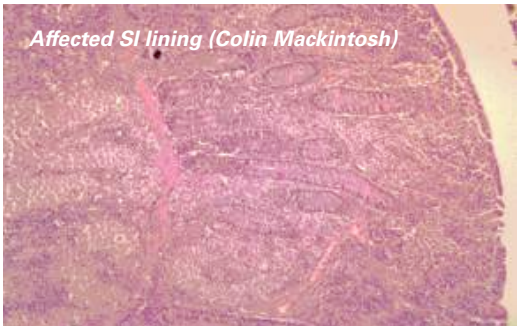
Johne's is a complex disease and combining the resources of the major livestock industry's and research partners to collaborate through the JDRC was an important step to coordinate and focus research investment to achieve the greatest benefit for New Zealand industry. The JDRC also had the benefit and privilege of being able to collaborate with and work alongside other research teams and industry bodies, both within New Zealand and internationally, to strengthen the outcomes from its programme and provide the best outcomes for New Zealand farmers.

Participants in the unincorporated joint venture were Beef + Lamb New Zealand Limited (B+LNZ), DairyNZ Limited (DNZ), DEEResearch Limited, AgResearch Limited (AgR), Livestock Improvement Corporation (LIC), Massey University and the University of Otago. The Meat Industry Association (MIA) and Dairy Companies Association of New Zealand (DCANZ) were associate participants in the Consortium. The Ministry of Business, Innovation and Employment (MBIE) provided funding to the Consortium via the Research Partnership funding scheme. Landcorp Farming Limited, Johne's Management Limited (JML) and The New Zealand Merino Company Limited (NZM) were also collaborators who invested in research with the JDRC.

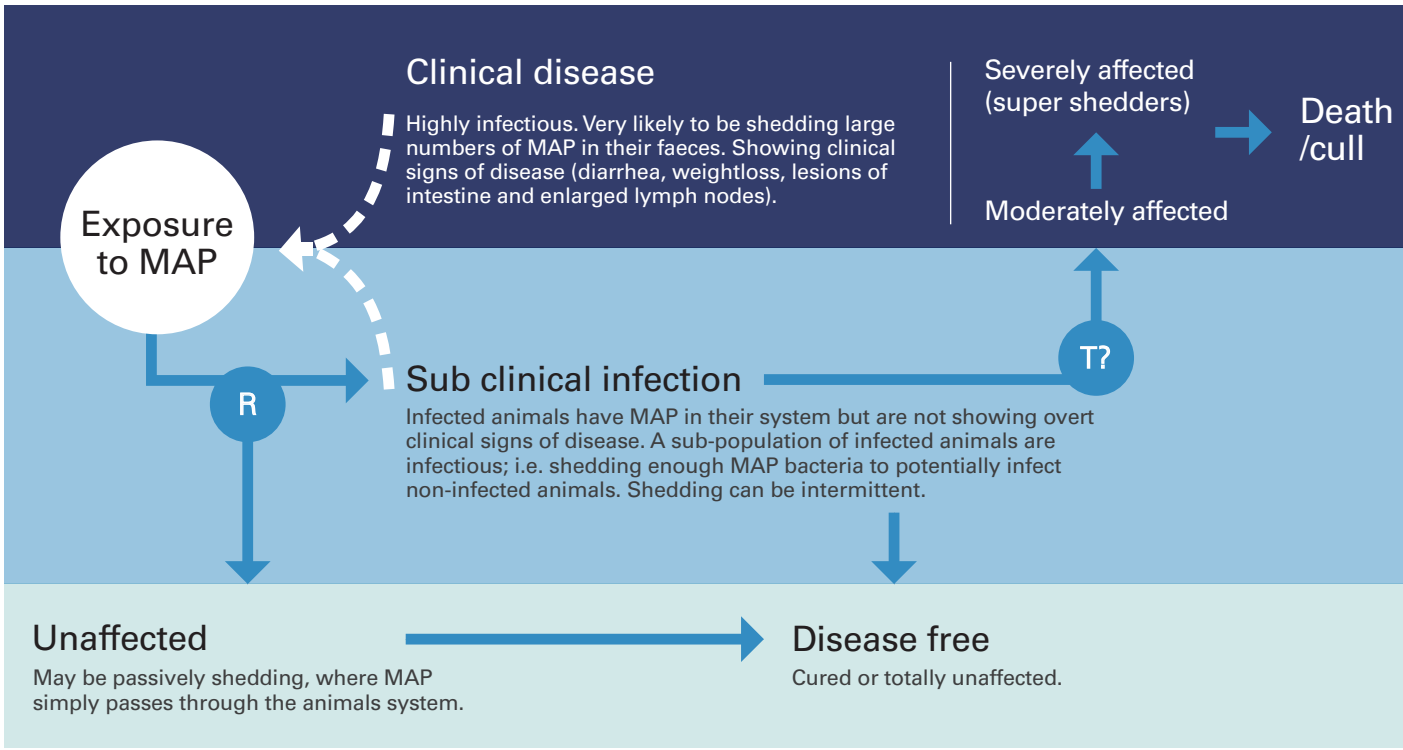
The disease

Johne's disease (JD) is a chronic, progressive, contagious and generally fatal infection of cattle, sheep, deer, goats and wildlife caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Infected animals contaminate the environment by shedding large numbers of MAP in their faeces, increasing the risk of infection passing amongst herds and flocks. Once infected, an animal can remain unaffected and show no signs of the disease throughout their lifetime, however a small number of animals progress to clinical disease. The bacteria cause an autoimmune reaction in the gut, thickening the intestinal wall and reducing the ability of an animal to absorb nutrients from the diet. Clinically affected animals suffer from wasting and eventually die from malnutrition. There is no recognised treatment for the disease. In New Zealand there are vaccines registered for sheep and deer which, while not preventing infection, will in most cases reduce the signs of clinical disease.


Normal SI lining (Colin Mackintosh)


Affected SI lining (Colin Mackintosh)

Pathways of MAP Infection



Impacts of the disease on farm

While results from research studies vary, evidence suggests that clinical JD affects animal production by reducing life expectancy, meat and milk yields and the value of cull animals. Sub-clinical disease may also affect production, but this impact is more difficult to measure. While the financial cost of JD is thought to be minimal on farms without clinical disease, the cost on the worst affected properties can be substantial and not limited to economic impact alone.

Data collected in 2014-15 by JDRC researchers suggests that the cost of Johne's disease to the New Zealand sheep sector is of the order of $75-92 million annually[1].

Data regarding the cost of the disease to other livestock industries is pending publication.

[1]Cord Heuer, January 2014

T?

It is not known what, or if "triggers" result in the progression of infection to clinical disease—but stress is one factor known to influence the manifestation of clinical disease in infected animals.

R

A spectrum of responses can be expected on exposure to MAP. Response will varydepending on species, strain of MAP, age of the host, size of the challenge, degree of stress affecting the host and the innate/acquired resistance of the host (resistance vs susceptibility).

Research programme

The aim of the JDRC research programme was to reduce the impact of Johne's disease on farm and involved a number of different approaches to disease minimisation.

The JDRC Science Plan was developed over a period of two years and included formal scientific and commercial reviews of all objectives by international experts.

Johne's disease is complex and MAP a difficult organism to study. The bacteria grow slowly in culture systems, are difficult to detect in the early stage of infection and their effect on ruminant animals usually long-term. The disease also manifests itself differently in different species which leads to a need for tailored management practices across the sectors. However, there are many common factors between the species and much to learn about how the disease behaves in New Zealand's multispecies, pastoral grazing environments, which improves our overall ability to manage the disease long-term.

The JDRC programme has invested research and development funds in four major areas:

- Diagnostics
- Pathobiology
- Genes and markers
- Epidemiology

The following sections detail the research and outcomes achieved in each of the four areas.

Dr Heinrich Albert Johne

SCIENTIFIC ADVISORY GROUP

The JDRC acknowledges the invaluable input of the Scientific Advisory Group (SAG), formed in 2008, to support the development and ongoing review of the JDRC Science Programme. The SAG was tasked with ensuring the scientific excellence, credibility and relevance of all research programmes and were unfailing in their support for the programme. The members of the SAG were:

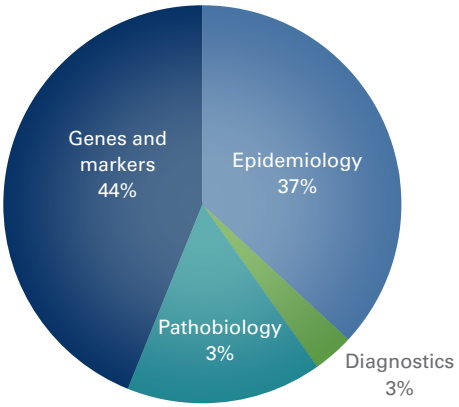
Dr Lindsay Burton—JDRC Science Manager and Chairman of the SAG

Prof Dirk Pfeiffer—Professor of Veterinary Epidemiology and Head of the Veterinary Epidemiology, Economics and Public Health at University College London

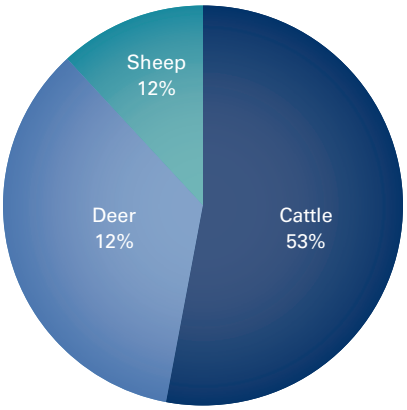
Dr John Bannantine—Research Microbiologist at the National Animal Disease Center of the USDA

Professor Stephen Bishop—Roslin Institute, United Kingdom. We note with sadness the passing of Stephen in April 2015 after a short illness.

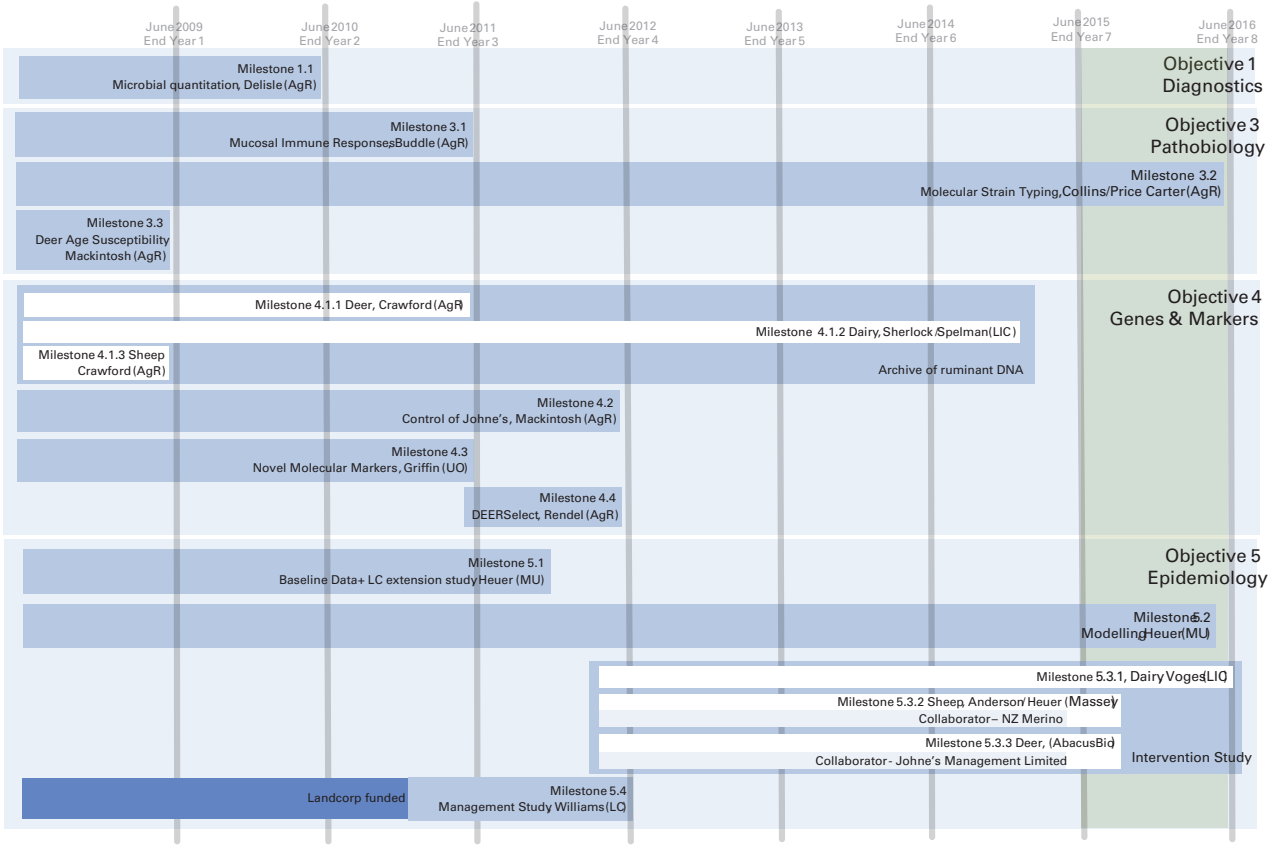
Science funding by objective



Species funding allocation



JDRC Science Plan Structure



Diagnostics

Objective

The original objective of the diagnostics milestone was to develop new diagnostic technology, to better characterise immunological responses in the target species of animals infected by *Mycobacterium avium paratuberculosis* (MAP), and to develop techniques for improved culture, molecular typing and characterisation of bacterial strains responsible for Johne's disease (JD). However, in the later stages of the program the focus of the diagnostic challenges was aimed at demonstrating parameters for the application of existing diagnostic tests for best use in New Zealand livestock.

Background

Diagnosis of MAP infection is challenging because of the nature of the pathogen and the slow progression of the disease.

The close relationship of different subspecies of *Mycobacterium avium* demand precise techniques to specifically diagnose MAP infection against a background of animal exposure to mycobacteria that are ubiquitous within the environment. The subtle differences in the phenotype of the "bovine" and "ovine" strains of MAP provide an additional level of complexity in researching these pathogenic mycobacteria.

Common diagnostic tests for JD and MAP infection either detect the presence of the Johne's bacteria (MAP) directly, or the host's immune response to the infection. The clinical stages of the disease are therefore relatively easy to diagnose as the animal is likely to be shedding bacteria in its faeces and to have mounted a significant immune response to the infection. However, in the subclinical stages of infection MAP can remain hidden in the gut wall for many years with little or no impact on the immune system and only intermittent shedding. The challenge for all researchers has been to develop methods better suited to diagnose subclinical infection, as the early identification of subclinically infected animals would greatly aid in controlling the spread of infection and reducing excretion and environmental contamination.

Summary of outcomes

Improving the ability to diagnose JD infection was the focus of early studies undertaken by the JDRC through research at AgResearch. Neither PCR nor quantitative PCR (qPCR) were routinely used as diagnostic methods for JD in 2008, with culture recognised as the "gold standard" for diagnostics. The JD work programme supported the development of quantitative methods for diagnosis of JD both by PCR and culture to aid in experimental studies, where an estimate of the degree of infection was required. While still used in some areas culture has now been largely superseded and qPCR recognised as the routine diagnostic test of choice for quantitation of MAP bacteria.

Work to improve diagnostic methods was also incorporated under the objectives of a number of other projects undertaken by the JDRC. In each of these projects researchers modified or provided data to validate existing technologies for the specific environment they were working in and improve diagnostic capability in New Zealand. Diagnostics were investigated in the following projects:

- **Pathobiology**—development of VNTR and SSR strain typing methods for New Zealand and initial investigation of Whole Genome Sequencing (WGS) technology
- **Dairy genetics**—development and validation of a methodology for ELISA testing for the purpose of screening dairy herds
- **Deer on-farm study**—comparison of Paralisa™ and Paracheck™ ELISA test methods and investigation of lesions in deer at slaughter.

The JDRC research programme did not attempt the development of new diagnostic techniques for MAP.

A clear focus of the diagnostic test work undertaken by the JDRC was to provide clarity for farmers and veterinarians on the correct application of available test methods for detecting disease. We have come to understand, based on the outcome of numerous studies both in New Zealand and internationally, that both ELISA and qPCR are very good tests for detecting clinical disease, but are not designed to identify sub-clinically affected animals. It is unlikely, based on current technologies, that we will be able to easily identify sub-clinical animals in the near future. However, when applied correctly, the existing tests can be used to identify animals that should be culled or managed to successfully reduce the levels of infection within a herd or flock. The JDRC has therefore worked with industry to provide understanding for those in the field about the application, utility and limitations of existing test methods to reduce confusion and increase confidence in testing methods.

Milestone 1.1

Microbial quantitation

Geoff de Lisle, AgResearch

Introduction

The microbial quantitation project was undertaken in the early stages of the programme to develop standardised procedures for quantifying the number of *Mycobacterium paratuberculosis* (MAP) bacteria present in liquid cultures and was carried out in parallel with the development of real-time quantitative PCR. At the time, the use of qPCR for MAP detection was in its early stages and not routinely undertaken in New Zealand.

Results and discussion

A series of investigations was carried out with liquid cultures and clinical samples to develop and evaluate methods for counting the Johne's bacilli, MAP. Their slow growth, propensity to form clumps and fastidious nutritional requirements made the accurate counting difficult, however these difficulties were overcome and a method was established for quantifying bacteria by culture. A real-time PCR test was also established through this test programme.

The quantitative measures obtained from this work were used to support a range of JDRC research projects, but the rapid establishment of qPCR as a commercial test for MAP by testing laboratories (particularly by Otago's Disease Research Laboratory, who are experts in PCR) supplanted the use of quantitative culture and the experimental PCR developed by AgR.

Pathobiology

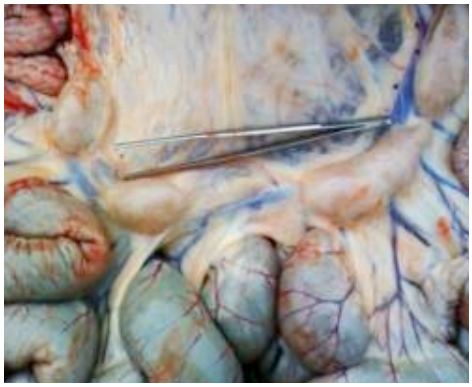
Objective

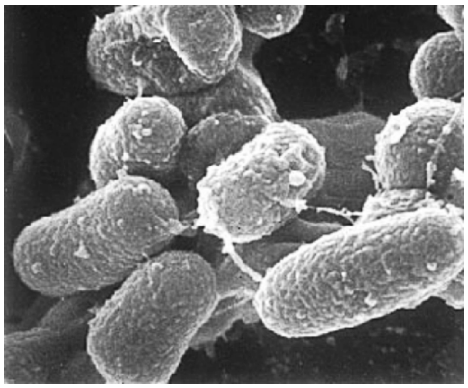
The objective of the pathobiology programme was to provide understanding of selected aspects of the complex interactions between the host, infectious organism and environment and to develop methods that would enable the successful accomplishment of other objectives.

Background

An understanding of the pathobiology of Johne's disease underpins many of the outcomes required for improving disease control, including better diagnostic tests, breeding for disease resistance, and herd management. Host/pathogen interactions determine what type of immune response is associated with disease, and knowledge of this assists in the development of improved diagnostic tests. Understanding the type of immune response associated with protection against disease helps define the requirements for markers for breeding for resistance (innate and acquired immunity). Understanding the different interactions that might occur between different strains of MAP and different hosts was a key component in determining some of the results from the epidemiology objective (page 31).

It was acknowledged in 2008 that our understanding of Johne's disease lagged well behind that of many other infectious diseases affecting animals. This was particularly evident in comparison to tuberculosis, which is also caused by a slow growing mycobacterium species. While Johne's disease resembles tuberculosis in some immunological and pathological features, it was more poorly understood and had inferior control tools, partly because the research was less advanced and partly because MAP is a sub-species of the *Mycobacterium avium* complex which is ubiquitous in the environment and cross-reacts with many tests. Not only were the basic aspects of paratuberculosis such as epidemiology, mode of infection, host-pathogen interaction, strain virulence, and immunology poorly understood, but the scientific tools available for controlling the disease such as diagnostic tests and vaccines were seen as inadequate for the purpose.





Top and centre: Enlarged lymph nodes and thickened gut of an animal with clinical disease.

Bottom: *Mycobacterium avium* subsp. *paratuberculosis.*

The JDRC programme was based on the premise that better control methods would most likely arise from results of further basic study of the disease to guide the applied science. This objective was therefore aimed at developing understandings and tools to enable the other objectives to be successful.

Summary of outcomes

The pathobiology programme provided three strands of fundamental work for the JDRC research programme. The most substantive part of the programme was the development of strain typing to investigate the nature of MAP infections in New Zealand. Both VNTR (variable number tandem repeat) and SSR (short sequence repeat) methods were developed and used to examine deer, sheep and cattle samples from across New Zealand and support our understanding of disease and disease transmission. With the rapid development of technology in this field VNTR and SSR typing has now been superseded by genome sequencing and the later stages of the JDRC programme were focussed on providing preliminary data on MAP lineages in cattle, sheep and deer.

In the second strand of work, a project identified the nature of gut immune response in cattle exposed to both experimental and natural MAP infection. Those animals that developed clinical disease failed to recognise MAP as a pathogen and mount the correct immune response to the disease. A number of genes were identified as targets for markers of disease susceptibility from this study.

The final strand of work established the relationship between age and disease susceptibility in deer; where it was found that younger deer were more likely to become infected and progress to clinical disease on exposure to the bacteria than more mature animals.

Milestone 3.1

Mucosal immune responses to Johne's disease in cattle

Bryce Buddle, Supatsak Subharat, Dairu Shu, Neil Wedlock, Geoff DeLisle, AgResearch

Introduction

The major site of infection for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the gastro-intestinal tract, yet prior to 2008 few studies had been directed towards this anatomical location. It was hypothesised that MAP subverted the mucosal immune system by activating regulatory networks or decreasing the expression of toll-like receptors (TLR) which recognise foreign pathogens to ensure its survival in the host. In this study blood and gut immune responses were compared in early stage infections established by experimental challenge of young calves with MAP and in cows naturally-infected with MAP. A secondary aim of the project was to establish a reproducible challenge system for calves with a pure culture of MAP to verify that specific gene markers correlated with disease susceptibility.

Method

In the experimental infection study twenty 5–8 week old calves were orally challenged with a culture of MAP and the infection monitored at two monthly intervals by faecal culture and systemic immune responses. A selection of animals were euthanized at seven months and the remainder at 15 months post-challenge and immunological studies undertaken on immune cells from blood and mesenteric lymph nodes (MLN).

In the cull cow study, 38 cows from herds infected with MAP were divided into four groups, based on MAP culture from gut tissues and histopathological lesion scores. Their immune responses in blood and mesenteric lymph node cells were measured.

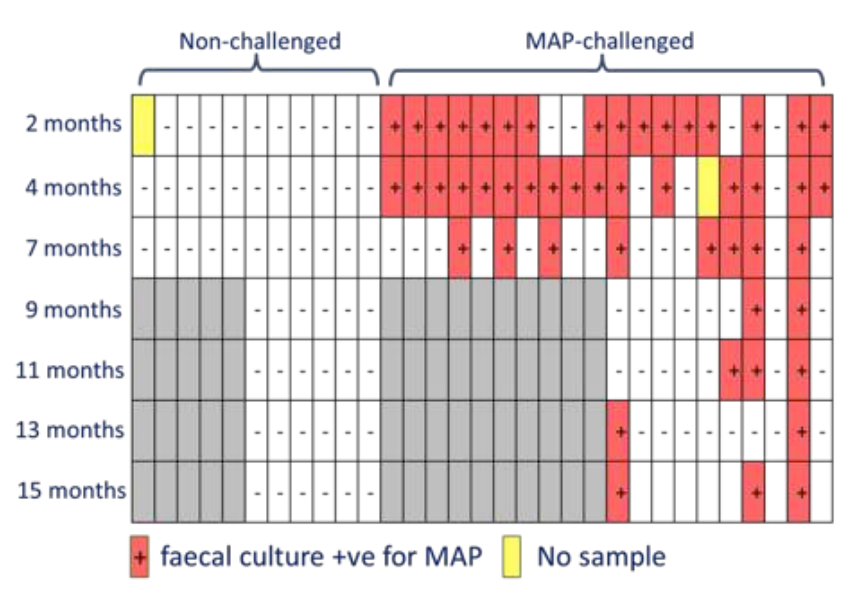
Results and discussion

Both the experimental challenge and cull cow study gave valuable information on systemic and mucosal immune responses of cattle infected with MAP. The outcome was identification of a number of diagnostic markers to identify MAP infection at early and late time points in the disease and genes to target for susceptibility studies.

The experimental challenge of calves with a pure culture of MAP developed in this study was shown to be an effective means of infecting calves. Of the 20 calves used, 19 were shedding MAP in their faeces between 2–4 months after challenge, indicating they had been infected with MAP. However, between 9–15 months post-challenge, a proportion of these calves controlled the infection and the number of animals shedding MAP in their faeces and with positive MAP cultures from their tissues decreased markedly. The severity of histopathological lesions also decreased. In the early stage of infection, 6–15 months after challenge, cellular immune assays from blood or lymph node cells were very useful for detecting MAP infection, but none of the animals were serologically positive.

For calves killed at seven or 15 months after challenge, their systemic and mucosal immune responses were similar, although the older animals had partially controlled the infection. The only difference seen was a failure of the toll like receptors (TLR) in younger calves to recognise MAP as foreign. TLR1 and two genes in cells from the calves cultured with MAP antigens were down-regulated, which is not the normal response to a pathogen. In the cull cows a disregulated immune response, typified by the induction of specific cytokine profiles and a failure to recognise MAP as foreign by TLR1 and two where shown to associated with the severity of disease.
The conclusion drawn was that these genes should be targeted for the identification of markers for disease susceptibility.

The study also noted that strong serological responses were associated with the presence of advanced JD and a large number of MAP in intestinal tissues, which is a potential source of infection for other animals. The severity of MAP infection in cull cows could be determined by MAP culture from tissues and histopathological lesion scores from gut tissues. Serological responses to MAP were only identified in cows with histological lesions.



Culture of MAP from faeces of calves 2–15 months post challenge

Culture of MAP from tissues of calves seven and 15 months post challenge
(mild histopathological lesions were observed, that were greater at seven months than 15 months)

Group	Months post-challenge	Mesenteric LN	Ileo-caecal LN	Distal Ileum	Ileo-caecal region
Control	7 (n=5)	0/5	0/5	0/5	0/5
	15 (n=6)	0/6	0/6	0/6	0/6
MAP–challenged	7 (n=10)	10/10	10/10	8/10	8/10
	15 (n=10)	6/10	4/10	3/10	3/10

Peer reviewed publications

Subharat, S., Shu, D., de Lisle. G.W., Buddle, B.M., Wedlock, D. N. 2012. *Altered patterns of toll-like receptor expression in cull cows infected with* Mycobacterium avium *subsp*. paratuberculosis. Vet. Immunol Immunopath. 145(1-2):471-8.

Shu, D., Subharat, S., Wedlock, D.N., Luo, D., de Lisle, G.W., Buddle B.M. 2011. *Diverse cytokine profile from mesenteric lymph node cells of cull cows severely affected with Johne's disease.* Clin. Vaccine Immunol. 18: 1467-1476.

Subharat S, Shu D, Wedlock DN, Price-Carter M, de Lisle GW, Luo D, Collins DM, Buddle BM. 2012. *Immune Responses associated with progression and control of infection in calves experimentally challenged with* Mycobacterium avium *subsp*. Paratuberculosis. Vet Immunol Immunopathol. 149(3-4):225-36.

Farmer interactions

Buddle, B.M. 2011. *Infectious disease and methane mitigation research in dairy cattle.* LIC Shareholders meeting, 5 July 2011, Hamilton.

Conferences and seminars

Buddle, B.M.; Shu, D.; Subharat, S.; Wedlock, D.N. 2010. *Evasion of protective immune responses to* Mycobacterium avium *subsp* paratuberculosis *in gut tissues of cattle.* 9th International Veterinary Immunology Symposium, Tokyo, Japan, 16-20 August 2010.

Subharat, S.; Shu, D.; Wedlock, D.N.; Buddle, B.M. 2010. *Mucosal immune responses Johne's disease in cattle.* IVABS seminar series, Massey University, Palmerston North, 24 September 2010.

Buddle, B.M. *Mucosal immune responses to Johne's disease in cattle.* VLA Weybridge, UK, 25 March 2011.

Subharat, S.; Shu, D.; Wedlock, D.N.; de Lisle, G.W.; Buddle, B.M. 2011. *Mucosal immune responses in cull cows with Johne's disease.* Moredun Research Institute, Edinburgh, UK, 28 March 2011.

Subharat, S.; Shu, D.; Wedlock, D.N.; de Lisle, G.W.; Buddle, B.M. 2011.
Dis-regulation of immune responses during early Mycobacterium avium *subsp*. paratuberculosis *infection of calves.* New Zealand branch of ASI annual scientific meeting. 30 June–1 July 2011, Wellington.

Milestone 3.2

Molecular strain typing

Marian Price Carter, Des Collins. AgResearch,

Introduction

The ability to distinguish strains of bacterial pathogens such as *Mycobacterium avium* subspecies *Paratuberculosis* (MAP) provides a basis for answering important epidemiological questions about sources of infection and the spread of disease and enables the investigation of the disease causing ability of different strain types for different hosts. Since 1980, the process of distinguishing or subtyping of MAP strains has increasingly relied on the direct detection of differences in bacterial DNA. Typing by Restriction fragment length polymorphism analysis (RFLPA) based on the insertion sequence IS900 typing clearly grouped strains of MAP into three clusters or subtypes. Strains of one group (called Type C or Type II) infect cattle and deer and are uncommon in sheep, while the other group(s) (called Type S or Types I and III), primarily infects sheep. While the IS900 assay has proven to be a very useful tool for understanding MAP bacteriology it lacked a level of discrimination necessary to answer questions about sources of infection and pathogenicity. The availability of large amounts of DNA sequence from mycobacterial genome projects in the mid-2000's led to the identification of repetitive DNA sequences known as variable number tandem repeats (VNTRs) and short sequence repeats (SSRs). When used for typing they gave a greater level of discrimination than IS900 and were relatively easy to carry out.

The objective of the molecular strain typing project was to provide a better understand New Zealand JD epidemiology and MAP pathology by developing a VNTR and SSR typing assay that could be reliably employed to differentiate between New Zealand subtypes of MAP in key samples from other JDRC projects.

VNTR and SSR DNA subtyping of MAP was an important new tool in 2008 because of its then superior ability to easily answer crucial epidemiological questions, and its usefulness in infection, vaccination and pathogenicity studies. By 2015 the technique had been largely superseded by advances in typing by whole genome sequencing (WGS), which facilitates a much higher resolution analysis of MAP genealogies and superior ability to assist with epidemiological studies.

Methods

Isolates of MAP were cultured in BACTEC medium containing egg yolk and the DNA was then extracted from the bacterial cultures for use in PCR reactions.

New Zealand MAP subtypes were characterised with VNTR and SSR loci that were identified in shotgun genome sequences that were available on public DNA databases. MAP DNA was subjected to PCR analysis, and PCR reaction conditions optimized for each VNTR locus.

For WGS analysis, MAP isolates were cultured initially in supplemented 7H9 media containing egg yolk, followed by culture on egg yolk free solid media. DNA was extracted and sent for sequencing at New Zealand Genomics limited. DNA libraries for these sequencing reactions were prepared using Nextera XT chemistry and the genomic libraries were sequenced on an Illumina Miseq platform. Genomic data were aligned to the K10 reference strain. Poorly covered regions of the genome were excluded from the analysis.

Results and discussion

In 2008–10, eight VNTR and two SSR loci that distinguished MAP subtypes overseas and five novel VNTR and five novel SSR assays that were developed at AgResearch were applied to a diverse set of archived isolates from cattle, deer and sheep from New Zealand and other sources. A subtyping system based on five VNTR loci and one SSR was developed and used as the basis for typing numerous isolates from New Zealand beef and dairy cattle, deer and sheep that were cultured for different JDRC studies.

Results indicated that there were eight different C types repeatedly detected, and that there was less diversity among New Zealand Type S isolates than Type C isolates, with only four S subtypes detected repeatedly. Most (89%) of analysed isolates were one of four MAP subtypes, one predominant C type carried mostly by cattle (but also sometimes by deer and sheep), a second less prevalent C type carried only by dairy cattle, a third C type carried by most New Zealand red deer (but also sometimes by cattle and sheep), and an S type carried by most New Zealand sheep (but also sometimes by beef and dairy cattle and deer).

Although the SSR8 assay (based on three DNA bases, TGG) is very helpful and reliable for subtyping New Zealand MAP subtypes, two SSR loci that monitor changes in the number of G bases at different chromosomal locations, which were helpful for subtyping MAP overseas (SSR1 and SSR2) did not reliably distinguish New Zealand subtypes.

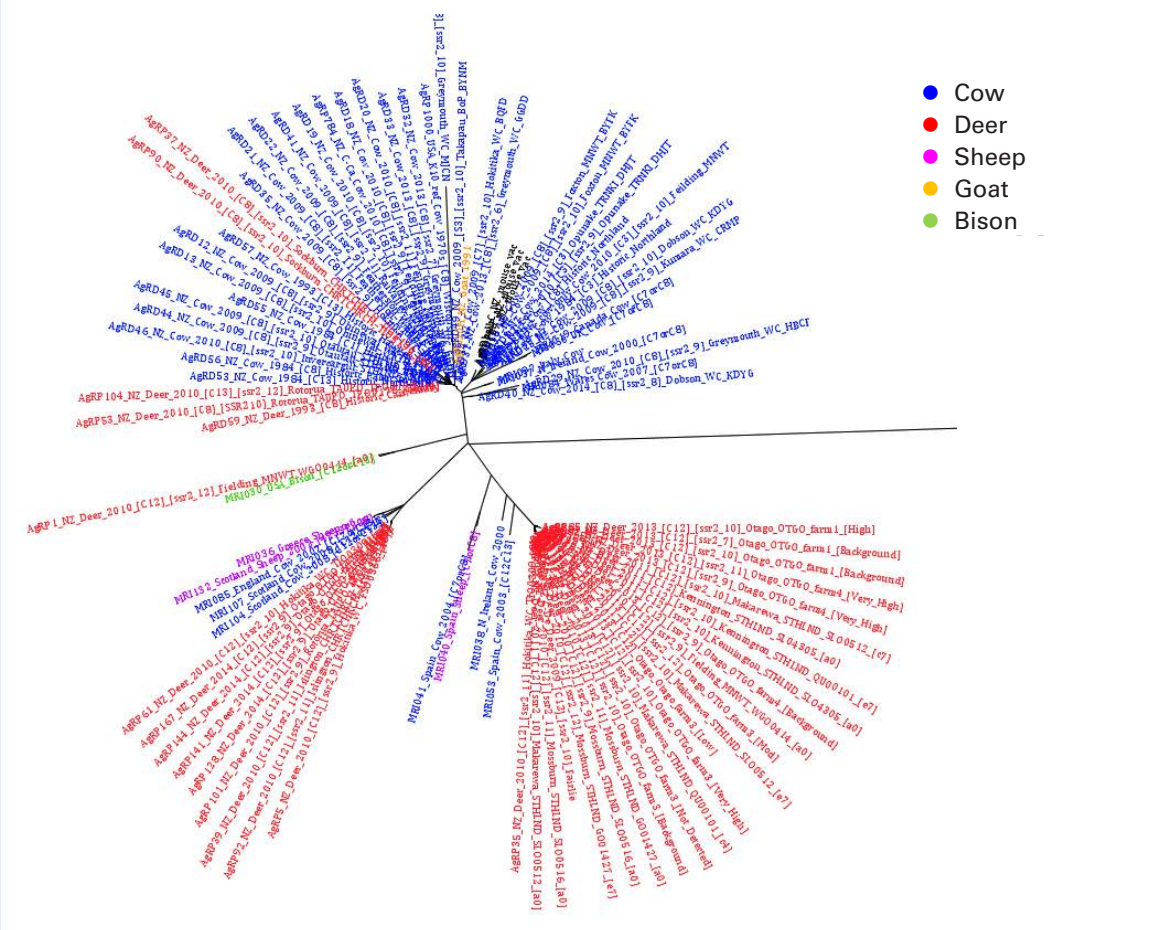
The following results were noted:

- The subtypes of MAP infecting livestock had changed significantly in the 15–20 years between when the older archived samples and more recent study samples collected. In particular, infection of cattle with Type S isolates was more prevalent in 2011 than previously determined and Type S isolates were more common in New Zealand beef cattle that were co-grazed with sheep than Type C isolates
- In highly infected dairy herds individual cattle were sometimes infected with more than one subtype of MAP
- Typing surveys of MAP isolates from deer lymph nodes with differing amounts of MAP bacteria and histopathology, faecal samples from deer that were shedding different levels of MAP, and faecal and lymph node samples from sheep with varying levels of OJD (as determined by serology and histology) conducted by both VNTR/SSR8 assay and WGS analysis, failed to reveal correlations between disease severity and MAP genotype. This failure may be the result of the small number of samples that were available to test or limitations of the methodologies, but also may indicate that disease severity is more likely to be influenced by host genetics and husbandry than MAP genotype

Preliminary analysis of SNP lineages by whole genome sequencing (WGS) has clearly shown that WGS is far superior to VNTR/SSR typing for distinguishing MAP isolates.

WGS analysis has revealed clustering of MAP subtypes on deer, dairy and sheep farms and promises to be a very useful tool for future investigations of New Zealand MAP epidemiology.

Relationship of C type New Zealand isolates by whole genome sequencing



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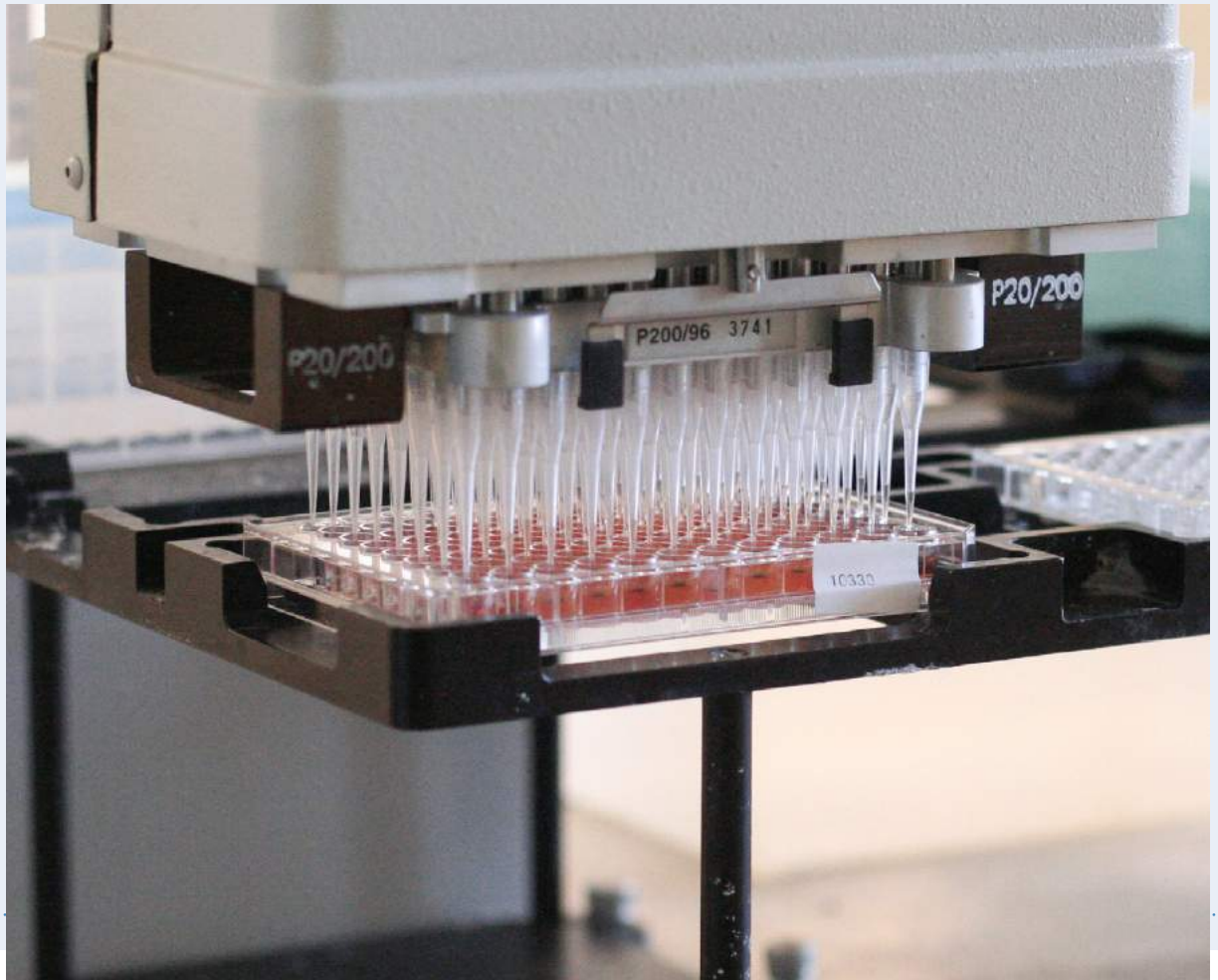

Milestone 3.3

Deer age susceptibility

Colin Mackintosh, AgResearch

Introduction

A three year study investigating the age susceptibility of deer to paratuberculosis, initially supported by the Foundation of Research Science and Technology funding, was completed by JDRC in 2009, at AgResearch, Invermay. Weaners, yearlings and adult deer were exposed to a heavy oral challenge of MAP and then monitored for signs of disease over 50 weeks.

Methods

Seventy female red deer in three different age-groups (30 three-month-old weaners, 20 yearlings and 20 adult hinds) received four oral doses of ~109 colony forming units (cfu) of a live virulent bovine strain of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in late March. They were closely monitored for the following 50 weeks and blood samples were taken periodically throughout the study for serology using the Paralisa™ test. Clinically affected animals were euthanised and the rest of the deer were killed at the end of the study. Necropsies were carried out on all animals and samples of jejunum (JJ), jejunal lymph node (JJLN), ileo-caecal valve (ICV) and ileo-caecal lymph node (ICLN) were taken for culture and histopathology from all the deer. Faecal samples were cultured midway through the study and at slaughter.

Results and discussion

Ten weaners developed clinical JD and were euthanised 20-28 weeks post-challenge (pc). No clinical cases occurred in the yearlings or adults during the 50 week study. Three weaners and one yearling died of incidental causes unrelated to the study.

The results indicated a clear age-related susceptibility of deer to the development of JD following heavy challenge with a bovine strain of MAP. Weaners, yearlings and adult deer were exposed to MAP and then monitored for 12 months following exposure to the organism. Results indicate that the disease is most severe in younger animals; clinical cases of JD only occurred in the weaners, and weaners were much more likely to be shedding MAP in their faeces than yearlings or adults. Almost all of the yearlings and adult deer became sub-clinically infected with MAP, but very few developed lesions in their intestinal tract characteristic of JD. This implies resistance to the development of clinical disease in older animals. It is hoped that understanding the relative susceptibility of different age groups to disease may assist the management and control of JD on infected farms.

Peer reviewed publications

Mackintosh, C.G.; Clark, R.G.; Thompson, B.; Tolentino, B.; Griffin, J.F.; de Lisle, G.W. *Age susceptibility of red deer* (Cervus elaphus) *to paratuberculosis.* 2010 Jul 14. Vet Microbiol. 143(2-4):255-61. Epub 2009 Nov 18.

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Genes and markers

Objective statement

The objective of the genes and markers work was to identify and validate a series of genetic markers that can be used to select Johne's resistant sires or screen commercial populations to identify resistant breeding stock.

Background

One of the most cost effective means of protecting pastoral species from the impact of diseases on farm is the development of improved genetics to cause an increased level of resistance across an entire population through centralised selective breeding technologies. Johne's disease (JD) is one of many infectious diseases which has been investigated to find genomic solutions to mitigate or eradicate the problem. The cryptic pathobiology and numerous animal factors that lead to resistant or susceptible pathology in Johne's were a significant challenge to be overcome in order to exploit a genomic solution in pastoral ruminants. There was also considerable cost associated with developing genomic solutions that was a barrier to the research. However, it was recognised that if successful the benefits of such a tool against the disease would be significant.

The identification and definition of reliable susceptible and resistant phenotypes was a key to success for genetic studies, therefore the sourcing and selection of accurately phenotyped stock representing clinically diseased animals was seen as important for all of the proposed mapping and expression studies. To assist in this a core part of the programme was the collection of DNA and tissue archives from which the genomic research could work with.

Summary of outcomes

In Dairy DNA was collected and genotyped from approximately 2000 cows affected by JD, identified by herd screening with ELISA tests. From this DNA Researchers identified a set of markers that were developed into a predictive test for susceptibility for Johne's disease (JDS). The test was licenced to and commercialised by Livestock Improvement Corporation in 2015. Validation studies have indicated that substantial work would be required to maintain the predictability of the test over time which has reduced its commercial viability.

In Deer a database of DNA from 1000 JD affected animals and matched controls was collected. This was not genotyped in 2010 due to high cost and concerns about route to market. However this database is being utilised in 2016 by the deer industry to support ongoing development of breeding solutions for JD in deer. The JDRC also developed a module for DEERSelect—the Deer Industry system—for evaluating the genetic worth of stags, to estimate breeding values for resistance to JD. This module was not implemented as it would have resulted in minimal gains for the industry compared with existing management techniques.

Deer were also used as the target species for the early stage investigation of novel markers for resistance and susceptibility from macrophages and for the purposes of a gene expression study which used DNA sequencing to identify genes whose expression was associated with resistance to JD. In both projects researchers successfully used cutting edge techniques to develop fundamental information about genetic markers.

Very limited work was undertaken by the JDRC around the genetics of JD in sheep. The programme initially sought to develop a DNA database, but it was difficult to find JD infected animals and an assessment of the nature and economic impact of the disease in sheep led to the conclusion that genetic solutions were not warranted and were unlikely to be economically viable for the sheep industry.

Milestone 4.1

An archive of ruminant DNA related to MAP infection for genome wide association studies in sheep, cattle and deer

Introduction

The objective of this milestone was to collect DNA samples from sheep, dairy cattle and deer to support the development of genome wide association studies (GWAS) in each species. In 2008 GWAS was a relatively new and expensive technology to look for markers of resistance and susceptibility to disease. The JDRC undertook to collect DNA for each species, and committed to GWAS studies for Dairy cattle, where implementation of any findings would find a ready path to market via established dairy breeding schemes.

Methods

Sheep

Samples for the sheep study were to be collected from JD suspect ewes during slaughter. Ileo-caecal valves and associated mesenteric lymph nodes showing gross pathology were collected from "skinny ewes" during processing at a meat processing plant in Southland. Control samples were taken from the "next in line" animal showing normal gross pathology. Samples were subject to tissue culture and the DNA isolated and purified.

Deer

Samples for the deer study were identified by Disease Research Laboratory (DRL), Microbiology, Otago University as part of routine herd testing undertaken by the laboratory. With permission from the farmer, blood from deer with a highly positive Paralisa™ test (PPA or Johnin score >100) was obtained in 10ml, heparin-containing, vaccutainers from DRL and the DNA purified by the method of Montgomery and Sise (1989).

Control samples were collected from age-matched deer in the same herd as the infected animals with no evidence of antibodies (PPA and Johnin score =0) in the Paralisa™ test.

Dairy

JD infected dairy cows were identified by milk-based ELISA test (USDA-approved IDEXX MAP Ab Test, based on Institut Pourquier technology). Ten animals were pooled together and if the pool was positive then individual samples were screened. Positive samples were verified by ELISA testing of a blood sample. DNA was recovered from verified samples and genotyping undertaken over Illumina high density (777K) marker panel. This was followed by statistical analysis with three different models, Plink, Bayesian Lasso and GeneDrop. Controls for the study were chosen from 25,000 animals genotyped through the LIC genomic programme.

Results and discussion

Sheep

The incidence of Johnes disease in poor-condition, cull-for-age ewes at the freezing works was found to be less than 0.3%, which provided insufficient samples to build a DNA archive for sheep. On review, it was decided that it was impractical and expensive to pursue a genetic based solution for sheep. The project was terminated and no archive built.

Deer

An archive of 2095 JD DNA samples was collected from 37 deer herds between 2008 to 2010. Johne's affected animals were identified by high Paralisa™ test results and the DNA isolated, purified and stored for future use. Phenotypic data was also collected and held stored in a secure database. In April 2016 the

DNA was taken out of storage and confirmed as viable for GWAS analysis and is currently being analysed for markers for resistance and susceptibility in deer (results pending). Should the work be successful the markers will be incorporated into the deer industry breeding scheme, DEERSelect.

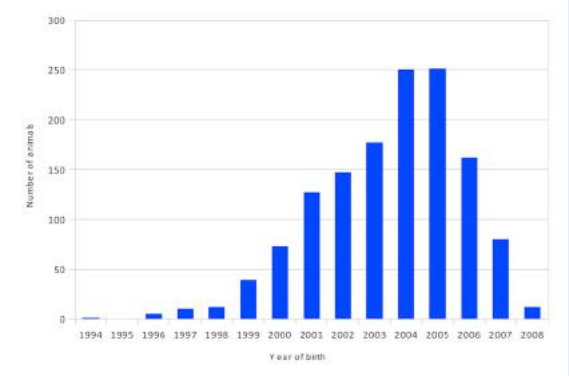
Dairy

DNA was collected from over 2000 dairy cows during the initial stages of the study.

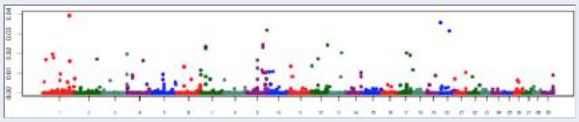
During the process to identify and collect DNA from affected dairy cattle, a range of phenotypic data was also collected and analysed for trends. This information suggested that JD was most frequently found in animals aged between 3–6 years old and more frequently in Jersey cows than Holstein Friesians. Over the production season 2009/10, JD positive cows in the study produced 47.1 kg milk solids less in 220 days than JD negative cows, which represents 14.3% of the mean. This value was similar to previously reported data for New Zealand herds (Norton 2007)

An additional outcome from the DNA collection work was the development of a bulk milk and blood ELISA testing service for JD by the LIC Animal Health team.

Of the >2000 DNA samples collected for the study, 1,833 Johne's-positive cows were genotyped on the HD panel and breed matched with 6849 cows from the control population. The data set was subject to multiple analyses to identify markers for susceptibility. Multi-SNP methods proved to be more effective for identifying signals than single SNP techniques. The results indicated that there were several regions of interest across the genome that had moderate association with the susceptibility of New Zeland dairy cattle to JD, from which a set of markers for JD were found.



Frequency of JD in dairy cattle with age of birth



Bayes effect variances for an association of single nucleotide polymorphisms (SNP), with Johne's status, from a multi-SNP, Bayes B genome-wide association study

These markers were developed into a predictability test for JD in cattle known as the "Johne's Disease Susceptibility (JDS) index". The index was licensed to LIC and made available to farmers via the 2015 LIC Alpha Sire catalogue. The reliability of the genomic prediction was considered low-moderate at approximately 30% and in all communications farmers were cautioned that use of JDS preferred sires in isolation would have only a small impact on the incidence of JD in a herd. The recommendation was that genomic solutions should be used in combination with a JD herd health plan to ensure maximum impact on disease control.

Subsequent testing to validate the JDS has shown that the test would require repeated and extensive re-testing to remain accurate over any period of time and therefore it is unlikely to be economically feasible for inclusion in LIC's ongoing breeding scheme.

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Milestone 4.2

Control of Johne's disease in farmed deer and cattle

Introduction

Two field studies were carried out in 2008/9 and 2009/10 in which weaner deer were challenged orally with virulent MAP. They had intestinal lymph node biopsies taken and were closely monitored over the 49–50 week study to identify animals that showed resistance (R) or susceptibility (S) to MAP challenge. A gene expression study was carried out on R and S animals to identify genes associated with resistance to Johne's disease (JD).

Methods

In the first field study 14 weaners bred by AI using semen from two stags that had been shown to be either resistant or susceptible to bovine tuberculosis were challenged with MAP.

At 4 and 12 weeks post challenge they were anaesthetized and a section of jejunal lymph node was surgically removed for culture (fresh tissue), histopathology (formalin fixed tissue), and genetic studies (snap frozen in liquid N).

In the second field study 18 weaners, which were bred from unselected hinds and sired by two stags known to be resistant (R) or susceptible (S) to paratuberculosis, were challenged with MAP and monitored for 49 weeks. Biopsy samples of the jejunal lymph node were collected at weeks four and 13 and at necropsy after euthanasia of clinically affected animals or when electively killed at week 49.

Results and discussion

All 14 deer in the first field study became infected but none were clinically affected. They all had varying degrees of subclinical disease when killed at week 50. Week 4 biopsies showed no paratuberculosis lesions, but MAP was cultured from all animals. At weeks 12 and 50 histopathological lesions ranged from mild to severe with corresponding low-to-high antibody titres, which peaked at 12–24 weeks.

The biopsy samples from the three most seriously diseased (designated susceptible "S") and the three mildly affected animals (designated resistant "R") were subjected to SOLiD SAGE (serial analysis of gene expression) next generation sequencing. This generated a total of 373 million transcript tags 26–28 bp in length after filtering. A total of 36,632unique transcripts were identified and 14,325 of these were able to be annotated. Copy number and differential gene expression analyses were undertaken on the data.

At four weeks genes significantly upregulated in R animals related specifically to host defence and all involve Type I interferon stimulated genes. By contrast genes upregulated in S animals related predominantly to inflammation, but also involved adaptive immune responses, mitochondrial function and apoptosis regulation.

At week 12, the genes upregulated in R animals were linked predominantly to regulation of adaptive immunity and mucosal immunity, while many of the genes in S animals were associated with pro-inflammatory interleukins involved with innate and adaptive immunity. These correlated with greater lesion severity and higher MAP numbers in lymph nodes of S animals.

By week 50 the number of upregulated genes declined in both groups. In R animals some appeared to be associated with host resistance and regulation of adaptive immunity. Genes upregulated in S animals involved antigen presentation and gut associated immune pathology.

It was concluded that, in this gene expression study following experimental MAP infection, a resistant phenotype was associated with pathways of adaptive immunity, while susceptibility was linked with upregulated non-protective pro-inflammatory responses.

In the second field study three animals (two S and one R) developed clinical disease and were euthanised. The nine S offspring had significantly more severe lesions than the nine R offspring. The average Lesion Severity Score (LSS) of R offspring was 5 (mild), and 7/9 had no or very mild lesions.

In contrast, the LSS of S offspring averaged 11 (severe), and 7/9 had severe lesions. Most of the resistant, but not the susceptible, animals showed evidence of resolving lesions and a reduction in the number of MAP between 13 and 49 weeks after challenge. One R offspring appeared to completely cure itself, and progressed from mild culture-positive paratuberculosis lesions at week 13 to having no signs of disease or infection 36 weeks later.

This study showed significant heritable resistance/susceptibility to paratuberculosis and key differences in immunological responses in the first three months after challenge, indicating different paths to relative success or failure to control MAP. In general, R deer had higher

IFN-ɣ levels, low antibody titres and fewer MAP, while S deer had lower IFN-ɣ levels, higher antibody and more MAP.

Peer reviewed publications

Mackintosh, C.; Clark, G.; Tolentino, B.; de Lisle, G.W.; Liggett, S.; Griffin, J.F.T. (2011). *Immunological and pathological responses of red deer resistant or susceptible genotypes, to experimental challenge with* Mycobacterium avium *subsp.* paratuberculosis. Veterinary Immunology and Immunopathology. Volume 143, pp 131–142

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Milestone 4.3

Novel molecular markers for identifying resistant phenotypes in red deer

Introduction

The primary drivers for this work were the existence of and access to purebred lines of deer which displayed polarised extremes of resistance or susceptibility to JD and a desire to determine what events occur post infection, within the host, to either limit or promote the uncontrolled proliferation of MAP and ensuing clinical disease. Some animals appear naturally more resistant to disease and this study was designed to look at the underlying mechanisms of resistance and therefore identify associated biomarkers that might be used to identify these animals. The molecular markers project was at the discovery end of the science spectrum and looked to use novel technology to identify genes differentially expressed by macrophage cells ex-vivo following mycobacterial challenge which could be used as markers of disease resistance and susceptibility.

Methods

Two groups of animals were used in this study. The first group comprised of twenty purebred, one year old red deer from a stud farm where the herd had experienced high levels of exposure to MAP. Within the herd where seven distinct deer breeds, some of which historically displayed extremes of resistance or susceptibility to JD. The experimental group included 10 animals that were considered to be of the susceptible genotype and 10 animals of resistant genotype which were all Paralisa™ negative and considered uninfected at the time of testing. The second group of animals comprised thirteen cross-bred animals with a predicted resistant or susceptible genotype based on historical breed and sire information. They were the progeny of an artificial insemination programme using semen from three stags. These animals were sampled at 5–6 months of age and at the time of sampling were all Paralisa™ negative.

Bulk blood samples (300 mL) were collected from all of the animals by jugular venepuncture and the samples used for gene expression and cell death detection experiments. Macrophages were cultured from the blood samples and infected with a K10 strain of MAP as described by Dobson et al. (2013). Next Generation Sequencing (NGS) technology was used to carry out an RNA-Seq transcriptome sequencing experiment using an Illumina HiSeq instrument using 100bp paired end reads on genes expressed from both MAP infected and uninfected controls.

Results and discussion

The initial phases of this project were technically challenging and required the development of new methodology to grow and isolate macrophages at a scale sufficient to provide large enough samples for interrogation by sequencing. Along with the successful development of methodology for assessing the immune response of animals to mycobacterial challenge and technology to measure differential gene expression of selected targets, a cervine transcriptome dataset was created from this work which would provide fundamental data about the response of macrophages to MAP infection.

Early analysis of this data set showed that the expression of most genes from the macrophages was altered on infection with MAP and that patterns of gene expression were different between resistant and susceptible animals.

It is noted that the JDRC funded the early stages of this project only (from 2008–2012) when the fundamental methodologies required to support the growth of macrophages and isolation of genes were developed and implemented by the team. While the work was technically successful and showing promise, the JDRC ceased funding this programme in 2012 as the focus of the

Consortium shifted to supporting R&D solutions that were closer to market. The work continued under the patronage of the New Zealand Deer Farmers Association (NZDFA) with co-funding from Callaghan Innovation. The outcomes from this ongoing research have been developed into a routine prognostic test for the relative scoring of JD resilience and susceptibility of sires. Validation of the test is currently the subject of a collaborative study between AgResearch and Disease Research Ltd in Otago, supported by NZDFA.

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Milestone 4.4

DEERSelect Module

Introduction

DEERSelect is New Zealand's national deer recording database which stores pedigree and performance records for the national herd. The data is used to provide estimates of breeding values and economic indices which a deer stud breeder can use to make selection decisions and monitor genetic progress within the nationally recorded herd.

The long term goal of this project was to provide tools to enable genetic gain for JD resistance by producing breeding values for deer sires. The first step was to create a module for DEERSelect comprised of progeny-derived breeding values (eBVs) from Johne's test results and performance data and make sire rankings available to farmers.

Methods

Data for the programme was provided from two breeders testing for Johne's using the Paralisa™ ELISA test and who were recording data on DEERSelect. These breeders had a total of four red herds and two Wapiti herds between them.

A total of 5,969 red deer (and 14,284 pedigrees) and 2,584 wapiti (6,945 pedigrees) were included in the analysis, with Johne's tests results recorded from 2006 to 2012 for the red deer and from 2007 to 2012 in the wapiti herds. All red herds had Johne's records on yearlings through to mixed age hinds. The wapiti herds had Johne's records for yearlings in only one year in each herd.

Data extracted from DEERSelect for these deer included information about birth flock, birth tag, birth year, birth date, sex, breed, Paralisa™ result, Paralisa™ test date, Paralisa™ herd, johnin titre, johnin test date, johnin herd, PPA test date, PPA titre, PPA test date, weights and weigh dates up to 24 months of age.

ASREML data analysis software was used to estimate genetic parameters. A Multi-trait model was used that included fixed effects for herd-year-sex, birth deviation, age of dam, and age at testing. Johnin and PPA were transformed to the loge scale for analysis. Correlations with liveweight traits were also investigated.

Results and discussion

Genetic parameters for measures of Johne's susceptibility were estimated from the data. They were moderate (0.16 to 0.26) and reasonably highly genetically correlated (0.85 to 0.94) in red deer. However, the heritability's were lower in the Wapiti. Genetic correlations with early liveweights (weaning weight, autumn weight, weight at 12 and 15 months of age) were generally low (-0.08 to 0.08).

A module was then developed for DEERSelect to estimate the breeding value for Johne's susceptibility for red deer. Initial estimates suggested the incidence of Paralisa™ positive animals would decrease by 0.3% to 0.7%per annum by including this trait in the DEERSelect indexes. When these reductions were considered against the reported 2–3%pa improvements that were being achieved using test and cull to remove high shedding animals it was considered there would be limited value in including the trait for JD in the DEERSelect index and the module was not implemented. It was concluded that the deer industry should focus on using other on-farm management strategies known to influence exposure and infection rates along with interventions (including testing and culling based on high quality laboratory tests results) to reduce the incidence of JD on farm.

References

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Rendel, J.M. and Newman, S.A. *The effectiveness and economics of Johne's disease test and cull in Deer.* AgResearch, May 2012. Confidential Report for Landcorp Farming Limited and Johne's Disease Research Consortium.

Epidemiology

Objective

The objective of this programme was to characterise and quantify the burden of Johne's disease on farms and to look for cost-effective management procedures for reducing production losses caused by Johne's disease. The focus was to better understand disease epidemiology, its economic impact and the relative importance of various sources and transmission pathways for the infection with *Mycobacterium avium paratuberculosis* in domestic livestock. The final stage of the programme was to test the ability and practicality of a range of interventions to reduce the levels of clinical disease on farm.

Background

Prior to 2008 there was very little data available on the nationwide prevalence of the MAP in livestock at both farm and animal-within-farm levels. Evidence suggested many, and possibly most, cattle herds and sheep flocks in New Zealand were infected with MAP and that there was a high prevalence of JD in the farmed deer population. MAP was also known to be present in a number of wild animal species commonly found on farms (rabbits, hedgehogs, possums).

In addition to there being multiple hosts for the pathogen in New Zealand there was also known to be multiple strains of MAP adding to the complexity of its epidemiology. This disease is multi-host and multi-strain with a large number of potential infection sources and transmission pathways. All of these sources and pathways potentially contributed to the level of JD in New Zealand livestock, but their relative importance was unclear. It was proposed that understanding these factors would help define feasible management tools for each species. Risk factors for disease included contact within the herd, with other species, contaminated pasture or water, hospital paddocks, wildlife.

The JDRC epidemiology programme was designed to provide information on disease prevalence, transmission and economics via on-farm work and computer modelling studies.

Summary of outputs

A substantial number of practical tools were generated via the epidemiology objective. This included the gathering of prevalence data for each species, understanding of risk factors for the transmission of JD and practical management tools for farmers as an outcome of on-farm studies.

Milestone 5.1

Baseline data

Cord Heuer, Massey University

Introduction

This project was designed to determine the herd/flock prevalence of MAP infection in single- and multi-species herds, determine risk factors for and herd/flock economic performance associated with the disease. Studies were also aimed at evaluating the likelihood of transmission between species within herds. Landcorp farming Ltd was a research partner with the JDRC in this study.

Methods

Prevalence study

A postal survey was conducted, followed by sampling of approximately 300 pastoral livestock herds in selected priority regions of New Zealand. Samples were analysed by ELISA, pooled faecal culture and then strain typed to provide evidence about transmission of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) between species (deer, sheep, beef cattle) and between farms, and associations between farm level infection status and production outcomes.

Data from the study was subject to latent class, stochastic analysis adjusting for (and evaluating) the lack of sensitivity and specificity of the tests employed. True prevalence estimates were adjusted for sampling fractions to reflect population prevalence.

Results

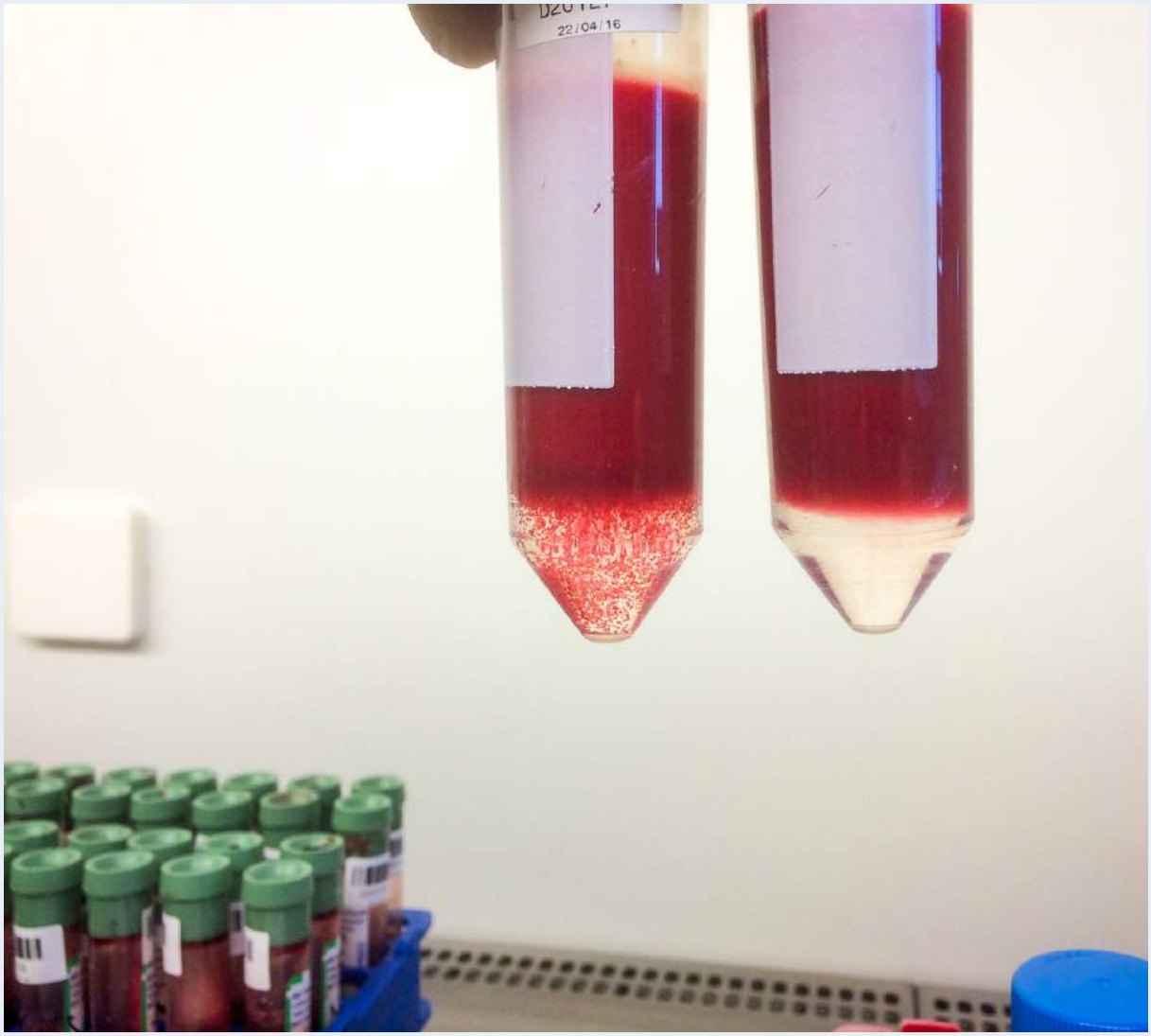
The core of the baseline data study was data from a postal survey and the analysis of samples from over 300 participating beef, sheep and deer farms across New Zealand. A total of 1,940 farms responded to the postal survey and 238 farms of these farms randomly selected for on-farm testing using pooled faecal sampling and serology (ELISA, Paralisa™). Samples were also gathered from a further 107 Landcorp properties.

The results from this analysis represent the best population based estimates of infection prevalence for JD currently available in New Zealand. It is estimated that 79% of sheep flocks (credible interval CI 71-86%), 52% of deer herds (CI 43-61%) and 44% of beef herds (CI 33-56%) were infected with MAP. In addition, 54% of Landcorp's dairy farms were found to be infected. While herd and flock prevalence was high, clinical JD occurred at very low rates on average (2–4 cases per 1,000 animals and year) whereas only very few farms experienced high rates (up to 6 per 100).

Strain typing of isolates collected in this study provided strong evidence for inter-species and between farm transmission. The results suggested that MAP is often transmitted between livestock species, most likely through contamination of jointly grazed pasture. Despite a relatively high level of farm prevalence, the transmission of MAP between farms was clearly evidenced by the analysis of movement data and DNA-strains.

The study results also provided data about the association between MAP infection and clinical JD, and farm productivity parameters. Lower pregnancy rates were seen in JD positive beef and deer herds, lower culling rates in JD positive beef herds, and higher culling rates in JD positive deer herds. Such associations require confirmation at animal level as confounding effects could not be excluded, but the outcomes suggest that alternate pasture management and animal husbandry may reduce negative effects of clinical and sub-clinical disease, livestock production performance and farm economics.

There was also evidence in the data of the between species transmission (within farm) impact of joint use of pasture. It was found that deer benefited from co-grazing with sheep but experienced higher infection and clinical disease rates when in contact with beef cattle. Beef cattle also increased the risk



for sheep, and were themselves at higher risk when in contact with sheep than when grazed in isolation. Both, beef cattle and sheep, benefited from the presence of deer.

A database of 345 livestock farms with known infection status, disease incidence and production performance was established alongside a bank of stored samples (faeces, blood serum). These are available for further studies about test validation, selection of JD affected farms for longitudinal and/or intervention studies.

Evidence from these three-year baseline studies lead to the conclusions that pastoral livestock are predominantly infected with MAP from pasture contaminated by either the same or different in-contact species, and that vertical or pseudo-vertical transmission may have less impact on the dynamics of JD than previously believed.

References

Heuer, C.; Wilson, P.; Larking, K. *Johne's disease in New Zealand Livestock.* VetScript 24(2), 39-41.

This objective disseminated information via the following:

- Survey results were communicated to client farmers of 28 veterinary practices
- Invited oral presentation at international (1) and national veterinary (7) conferences
- Nine publications at international conferences
- One journal publication (Vet.Pathology).

Milestone 5.2

Modelling

Cord Heuer, Cristobal Verdugo, Nelly Marquetoux

Introduction

The objective of the modelling work was to develop mathematical models of Johne's disease (or Paratuberculosis, PTB) dynamics over time. The first phase (2008-2011) focussed on developing single species models of prevalence and incidence, the second (2012-2013) extending the models to combine two host-species (sheep/cattle) and two MAP strains and evaluating intervention measures and in the third and final phase determining the cost effectiveness of interventions for sheep.

Methods, results and discussion

Diagnostic prediction model (31 December 2009)

Faeces and blood samples were collected from individual deer from 20 herds in the South Island and 18 herds in the North Island with an approximately equal representation of farms with/without observed clinical JD. Samples were tested by individual faecal culture and Paralisa™. A diagnostic model for deer was developed to evaluate the accuracy of faecal culture and Paralisa™ as tests for MAP infection in apparently healthy 1+ year-old deer. A latent class diagnostic model was developed in WinBugs through collaboration with the University of California, US, and University of Warwick, UK.

[Veterinary Diagnostic Investigation, 25(6), 759-764, 2013].

First prototype of a transmission model, within species (31 March 2010)

A state-transition model was developed that includes a susceptible (state S), an age-dependent resistant (state R), and a bifurcated (slow 's' or fast 'f' track) latent to infectious state (states L_s/f and I_s/f). Parameters were modified to fit simulation results to both experimental data and survey estimates of prevalence and incidence. Horizontal transmission depended on dry matter intake (DMI), the colony forming units (CFU) of MAP organisms on pasture, and the transmission parameter which encompasses both rate of contact (between susceptible animal and the environment) and infectiousness of contacts.

[Preventive Veterinary Medicine, 106(1), 63-74]

Transmission model, between farms (31 December 2011)

Modelling the transmission of MAP between farms was based on four years of movement data from 103 properties of Landcorp Ltd., the largest corporate farm enterprise in New Zealand. The techniques used for this purpose were Social Network Analysis (SNA) of farms connected through livestock movements, and molecular strain typing. The likelihood of two farms sharing the same genetic MAP strain was regressed on the number of network 'paths' by which two farms were connected. The model provided strong dose-response evidence for effective MAP transmission between farms through movements of livestock.

Model of economic effects of on-farm control measures (31 December 2012)

Early detection of young deer with clinical JD was effective for reducing prevalence and likely financially attractive (due to low cost). On the contrary, test and cull (T&C) using an ELISA did not reduce prevalence. In this simulation T&C was neither efficient nor cost effective. The model showed that seasonal variation of MAP survival on pasture had little impact on transmission dynamics, and that rotational grazing with pasture spelling versus permanent grazing of the same paddock reduced both infection prevalence and clinical JD by about 50%.

[Preventive Veterinary Medicine, 106(1), 63-74]

Effect of intervention scenarios on infection prevalence and clinical JD presented (30 June 2012)

Two models (deer and sheep) were developed and presented. Results suggested that two MAP genotypes tend to coexist in deer only if pathogen virulence was similar (+/- 10% virulence), else a less infectious genotype would be out-competed. A systematic literature review and a meta-analysis were carried out to define infection parameters for the sheep model. The parameters for this model were set to mimic seasonally limited events of birth, removals and replacements of sheep. The model was able to reproduce production and reproduction performances comparable to typical New Zealand sheep farms.

Mixed species sheep-cattle model developed and presented (31 December 2012)

A model of JD in a mixed sheep&beef (S&B) farm was developed and evaluated. Co-grazing two infected species, either set-stocked or by rotational grazing, increased JD infection prevalence to a higher level than on single species farms. When set-stocked around calving/lambing for four months per year, naive sheep acquired infection from infected beef cattle through grazing infected pasture faster and with higher prevalence, than naive beef cattle being infected by sheep.

[PhD Thesis C. Verdugo, 2013]

Cost-effectiveness of intervention scenarios evaluated and presented for single species farms (31 December 2013)

Sheep: When vaccinating replacement lambs at weaning, the sheep model has shown that the annual clinical incidence of JD (i.e. OJD mortality) would start to decrease from 2.7% two years after starting to vaccinate and reach zero at about 9–10 years. This suggests that farmers need to maintain vaccination for an extended period before they would realise production benefits. The model further suggested that positive returns over investment were achieved if JD caused 1.8% mortality whereas 0.75% mortality would not render vaccination as cost-effective. Therefore, the critical economic threshold for vaccination was an OJD related mortality of about 1–2%.

Sheep and beef: on mixed sheep and beef farms, interventions to reduce JD were more effective when applied to both species. Where whole herd/ ELISA based T&C effectively reduced prevalence in beef cattle, it did not do so in sheep. Moreover, the model indicated that (T&C) would need to be undertaken for 10 years before a notable prevalence decrease was achieved in beef cattle. Based on the high cost, in this scenario, T&C is unlikely to be effective. However, faecal RT-PCR based T&C may be more efficient and economical. Farmer surveillance appeared to be more effective in sheep than in beef cattle. Pasture spelling periods of more than six months controlled new infection rates by about 25 years after inception. Due to the slow effect and relatively long spelling periods, this does not seem to be an effective means of JD control. Grazing sheep and beef cattle in isolation from each other could not control JD prevalence when both were initially infected. However, in combination with increased farmer surveillance, species isolation reduced the prevalence rapidly in sheep but only quite slowly in beef. However, this combination was the most effective intervention for both species.

[PhD Thesis C. Verdugo, 2013]

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Stringer, L. A.; Jones, G.; Jewell, C. P.; Noble, A. D.; Heuer, C.; Wilson, P. R.; Johnson, W. O. (2013). *Bayesian estimation of the sensitivity and specificity of individual fecal culture and Paralisa to detect* Mycobacterium avium *subspecies* paratuberculosis *infection in young farmed deer.* Journal of Veterinary Diagnostic Investigation, 25(6), 759-764.

Heuer, C.; Mitchell, R. M.; Schukken, Y. H.; Lu, Z.; Verdugo, C.; and Wilson, P. R. (2012). *Modelling transmission dynamics of paratuberculosis of red deer under pastoral farming conditions.* Preventive Veterinary Medicine, 106(1), 63-74.

Heuer, C.; Wilson, P. and Larking, K. (2011). *Johne's disease in New Zealand livestock.* Vetscript, 24(2), 39-41.

Milestone 5.3.1

Dairy intervention study

Hinrich Voges (LIC)

Introduction

A four year intervention study for dairy cattle was instigated in 2012 which was designed to develop and refine practical and cost-effective risk management tools JD for the dairy industry in New Zealand. The aim was to provide a toolkit to outline best practice risk reduction steps recommended for problem herds (i.e. 1–5% of the national herd) and interventions to minimise transmission rates in low prevalence herds.

The study was comprised of case-control component to better define risk factors for JD in New Zeland dairy herds and thus refine the management interventions to test, an intervention trial amongst a small number of focus herds principally to study implementation of recommended risk reduction strategies to minimise transmission of MAP and an observational trial to monitor infection levels in a large number of JD herds with voluntary implementation of the risk management toolkit.

Methods

The Case-Control Survey was carried out using a two-tiered approach, starting initially with a phone survey, followed by enrollment in a postal/email survey on agreement from the farmer. Approximately 1900 respondents participated in the phone interview out of 2400 herd owners selected for trial. Over half agreed to receive the extended written questionnaire which was mailed from LIC to the participants and of those 551 forms were returned completed. The questionnaire was divided into nine sections, including the history of JD in the herd and herd replacement policy, lactation, evaluation of herd specific management of calving, weaning and heifers. The data was collated and subject to statistical analysis.

For the Intervention Study potential candidates were selected from bulk milk vat ELISA test screening results or previous expressions of interest by herd owners and/or their veterinarians where these herds had screened their cows by pooled herd-test JD ELISA for culling/risk reduction. All the selected herds had identified Johne's disease as an issue and were looking for interventions to reduce the impact. Owners were contacted at the end of May and enrolled before the start of calving. Herds were sampled for JD prevelance and a control plan developed with the herd manager and their vet, aimed at reducing the transmission of MAP by good calves and heifers management. Each herd was visited at least twice during the study period and was subject to annual herd screening to test for JD.

In addition to the main intervention herds, a second pool of herds and cows were enrolled in the study from corporate herds of LandCorp and Synlait. These farms implemented control strategies on a voluntary basis and were subject to annual whole herd testing using pooled-individual herd-test milk ELISA of these herds which was subsidised by the JDRC. Results were used to inform culling decisions to reduce calf exposure to MAP on farm and information from these herds used to enlarge the data set for the study.

Results and discussion

Case-control survey

Four hundred and fifty-seven study farmers participated in the final case study. Two hundred and forty-eight farmers (54.3%) had suspected or diagnosed JD in at least one dairy cow in their herd, with a mean and median within-herd incidence of 0.47% (S.D. 0.76%) and 0.20% (min: 0.0%; max: 6.2%), respectively. One hundred and forty-eight farmers (32.4%) reported a within-herd incidence of JD of ≥0.5%. Two hundred and nine farmers (45.7%) reported no cases of JD since 1 January 2012.

The mean herd-level presence and within-herd incidence of farmer-diagnosed JD was higher in South Island study herds relative to North Island herds. However, the highest within-herd incidence of JD observed/diagnosed (6.2%) was reported by a study farmer located in the Waikato region. There was an apparent seasonality in the timing of herd-level JD diagnosis/suspicion, with peaks in September and March. All reported cases were found in heifers or cows (there were no cases in bulls or steers). Ten farmers (5.9%) reported observing JD in heifers (rising 2-year-olds), while the remaining reported cases were adult cows ≥3 years of age.

The aim of the case control study was to look at the changing trends in dairy herd management in New Zealand and identify any specific risk factors affecting dairy herds. Data from the case study herds was therefore subject to statistical analysis to identify risk factors for farmer diagnosis of JD in a herd. The results suggested that South Island herds are more likely to be affected by JD than those in the North and that increasing herd size is directly correlated with increased JD risk. With rising average herd size in New Zealand (40% increase in over the last 10 years) measures targeting MAP transmission are considered to be important especially for farms in the South Island where the average herd is twice the size of North Island herds. The case-control study data also showed a highly significant association between the time that calves remain on the dairy platform/ home farm and JD risk. This result directed the focus of control strategies on protecting the replacement heifers beyond the calving and pre-weaning period.

Intervention Study

The 20 farms were enrolled in the intervention study early in 2012 for the purpose of investigating the effectiveness and practicality of intervention strategies designed to reduce MAP transmission, with a specific focus on calves and heifers. These herds ranged from small to very large, both individually and large corporate owned herds.

The interventions trialled in these herds were grouped into five overall strategies aimed primarily at protecting the young calves and replacement heifers:

1. Culling high-risk animals (includes testing)
2. Calving
3. Pre-weaning heifer management
4. Post-weaning heifer management
5. Herd biosecurity.

New Zealand herds that are affected by JD have a wide range of management practices and varying capacity to implement interventions. It was therefore essential to provide herd owners and their vets a range of solutions that could be applied as appropriate. The interventions chosen for the study were presented in three categories:

- **Best practice.** The ideal scenario for herds suffering significant JD losses, but which may be costly or unnecessary in herds that do not have Johne's disease problems
- **Alternative options.** Options that go some way to mitigating the risks
- **High-risk behaviours.** Practices that favour MAP transmission and should be avoided by all herds.

In mid-2016 it was noted that it was too early to see the long term effect of protecting replacement heifers against exposure and infection by implementing these JD strategies, but this set of initially highly affected farmers were noting the benefit of pre-calving test and cull through a dramatic reduction in the number of clinical cases during the season (including a number who recorded no cases of clinical disease).

The interventions trialled on these herds were developed into the "Dairy toolbox" for JD and published by DairyNZ in 2015.

References

Hunnam, J.; Cognosco, A. *Risk factors for the presence of Johne's disease in dairy herds: a survey of New Zealand farmers; Final Report.* Animal Health Centre, 8 April 2014

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Dairy toolbox, DairyNZ website. www.dairynz.co.nz/animal/cow-health/johnes-disease/

Milestone 5.3.2

Ewe death study

Cord Heuer (Massey University),
Peter Anderson (Marlborough Veterinary Centre)

Introduction

The primary goal of this research was to estimate the production loss caused by Ovine Johne's disease (OJD). A secondary objective was to evaluate the likely financial benefit of vaccinating against OJD.

The research was partially co-funded by the New Zealand Sheep Industry Transformation Project, NZSTX—a Primary Growth Partnership programme led by The New Zealand Merino Company and co-funded by the Ministry for Primary Industries.

Methods

Farm-level prospective monitoring was undertaken on 20 farms voluntarily participating in this study from August 2012 through to March 2014. Over that period sheep tallies were collected at mating, scanning and tailing/weaning. Deaths of lambs and ewes were calculated from animals present at these times minus removals (sales) plus additions (purchases). Results of scanning and tailing percentages and lamb and ewe loss were obtained. During the study ewes with a low body condition (BCS) were identified by the farmer. The most affected ewes that they suspected as being clinically affected with OJD were euthanised and subjected to post-mortem examination (PM). This data and the information from tallies was used to estimate overall OJD mortality rates within these flocks.

Those ewes with a low BCS where the farmer was unsure of the cause were labelled as "OJD suspect" and were blood sampled for ELISA testing. If these ewes did progress to clinical disease they were subjected to PM, but if they did not die and remained in the flock, the ELISA positive ewes were regarded as being sub-clinically affected.

Results and discussion

Of the 20 farms participating, valid data was obtained from 17 "fine wool" properties and the remainder from coarse wool breeds. This was the first time that a measure of ewe loss had been taken for a full year from mating to mating. Previously annual ewe loss tallies have been based on the difference between mating or scanning tallies and weaning tallies which misses 4–6 months of a year. Obtaining accurate tallies was a challenge for this work and relied on on-farm data verification and a high level of enthusiasm by the participants.

Performance was monitored in over 100,000 ewes on the 17 farms, with 390 PMs carried out by 12 veterinarians. It is noted that all of the participating farmers and the veterinarians had an interest in OJD and therefore the properties were regarded as farms affected by OJD to a greater extent than a "typical" New Zealand farm. This will have introduced a certain degree of bias to the study.

The average annual mortality rate of mixed aged ewes on these farms was between 7–8%. This included 0.7% of deaths in coarse wool breeds and 2.8% of deaths in fine wool breeds being caused by JD. The ewe death rate due to OJD was estimated from the overall ewe mortality, the proportion of ewe deaths observed by the farmer that were potential OJD cases and the proportion of ewes with confirmed OJD by PM examination. There was a large variation in the rates of OJD seen farm to farm, but the overall mortality rates were similar on each property. While OJD morality rates were high, a number of other conditions were also causing wasting and ewe death on these properties, including parasites, poor molars, poor legs or feet, enteric and respiratory disease.

Production loss on farm was estimated from both the impact of clinical and subclinical disease on farm. Rates of subclincal disease on these farms varied between 7–35%, which while reducing the productive lifetime of a ewe by between 0.5–1.3 years, was considered to have a minor impact on production when compared to the losses incurred through deaths due to clinical disease. The estimate of total production loss due to OJD in New Zealand was in of the order of $19–36 million annually. A number of assumptions were required to calculate this figure given the biases and limitations of the original data set.

The final output from the study was an estimate of the cost-benefit of vaccination as a control tool for OJD. Gudair® is a one-shot for life vaccine that reduces the incidence of clinical OJD and shedding in flocks. Some farms in the study had used the vaccine for their replacement ewe lambs in some years. Assuming a vaccine efficacy for preventing clinical illness reported from Australia, a benefit/cost ratio for the marginal difference between vaccinated vs. non-vaccinated flocks was estimated. Results from this study indicate that vaccination would be cost effective if farmers were noting clinical cases of OJD in their flocks above an approximate threshold of clinical disease over one per 100 ewes and year (>1% annual mortality).

References

Anderson, P. and Heuer, C. *Ewe mortality and Johnes Disease—Potential benefits from monitoring OJD in fine wool flocks.* Conference Proceedings, New Zealand Veterinary Association, June 2016.

Milestone 5.3.3

Deer intervention study

Peter Fennessy and Neville Jopson (AbacusBio Ltd), Solis Norton (Johne's Management Limited)

Introduction

The on-farm deer study had three separate aims.

The first was to compare the performance of two serum ELISA tests, Paralisa™ and PARACHEK®2, for detecting deer shedding high numbers of MAP. Blood testing with Paralisa™ is often an integral part of JD control in New Zealand deer herds[1]. It is relatively low cost and when used to guide health management decisions, can be successfully used to reduce the rate of clinical disease in herds.

However, reliance solely on the Paralisa™ was considered a potential risk for the deer industry. Therefore, this study sought to assess a second commercially-available serum ELISA (Prionics PARACHEK®2[2]), an earlier version of which had been applied to deer, as a potential alternative to Paralisa™. The assessment was undertaken under testing conditions commonly used by deer farmers and veterinarians seeking to control JD in deer.

The second aim of the study was to validate the national deer slaughterhouse surveillance database administered by Johne's Management Limited (JML). This database contains individual animal level information on all processed farmed deer. In particular it records lymph node enlargement and lesions indicative of Johne's disease (JDSLN) identified during routine carcass inspection by AsureQuality meat inspectors. The relationship between farm-level rate of these JDSLN and farmer reported JD-related death rate for that farm was assessed, as well as farmer concern regarding the disease. The incidence of JDSLN at the farm level was one of the tools used to prioritise farms for contact and support by JML. However, the relationship between JDSLN and the severity of the disease on-farm had not been formally determined.

The final aim of this study was to provide case studies and advice to industry regarding the best use of diagnostic tools for JD in deer.

Data from the study were to be evaluated and then presented to industry to ensure effective transfer of the findings back to industry.

The study was co-funded by Johne's Management Limited.

Methods

Diagnostic comparison

Infected herds were selected for sampling based on the following criteria:

- They had a JDSLN rate >1%
- They were reporting a significant number of deaths or a tail end of slow-growing or clinically-diseased animals, or
- The herds had a non-specific TB reactor problem.

As a first step, rising two year old hinds (R2) in the selected herds were screened using the Paralisa™ blood test to identify infected animals. Those animals that tested Paralisa™ positive, plus a random sample of Paralisa™ negative deer in these herds, were then tested using both the Paralisa™ and PARACHEK®2 blood tests and faecal qPCR. Sensitivity and specificity of Paralisa™ and PARACHEK®2 were calculated relative to faecal qPCR results at shedding thresholds of greater than 102 to greater than 107 organisms per gram of faeces.

[1] Griffin, J.F.T.; Evelyn Spittle, E.; Rodgers, C. R.; Liggett, S; Cooper, M; Bakker, D; Bannantine, J. P.; Diagn C. *Immunoglobulin G1 Enzyme-Linked Immunosorbent Assay for Diagnosis of Johne's Disease in Red Deer* (Cervus elaphus). Lab Immunol. 2005 December; 12(12): 1401–1409. doi: 10.1128/CDLI.12.12.1401-1409.2005

[2] www.thermofisher.com/nz/en/home/industrial/animal-health

JML validation study

The validation study was completed via a telephone survey conducted by JML staff in two distinct parts. The first part of the survey assessed JD-related productivity loss based on the farmer's estimate of the number of deer that either died or were euthanased due to JD, and their valuation of this loss. It collected information on the profile of farms including size, type of deer, enterprises, deer purchases and sales, and farmer experience with deer farming and social farming networks. It also included questions relating to the incidence of JD and the perspectives of the farmer in relation to JD.

The second part of the survey used 1000Minds® software to determine farmer's perceptions of the impact of JD on their farms and to analyse how farmers perceive the cost of JD on their farms. With 1000Minds® methodology, the respondent is asked to choose between similar pairs of alternatives, all with the same basic outcome, to analyse the farmer's perception of the consequences of JD in terms of economic impact, deer deaths and reduction in weaning rate.

Results and discussion

JML validation study

A total of 151 farmers participated in the survey. Their farms were selected to cover a range of JDSLN rates from zero to high (>2%) based on data from 2010 to 2013.

There was a general high level of concern about JD in the studied farms with 35% of the farmers being highly concerned about the disease and less than 10% having no concern. Overall farmers preferred to see less JD-related killings and more dollars from the processor rather than higher weaning rates, although the economic implications of the three alternatives were designed to be exactly the same within the survey.

When considering the relationship between JDSLN rate and JD-related death rate the results where indecisive, with neither a positive or negative relationship evident in the data. However, farmers with a high degree of concern about the disease tended to have a high JD-related death rate in their deer on-farm and/or a high rate of JDSLN in their processed deer. The practical implication of this is that the JML programme should continue to utilise multiple methods to prioritise farmers for contact and support in controlling JD and should not rely solely on JDSLN rate.

Two other noteworthy results were revealed. Only 8/151 (5%) respondents felt they had not seen signs of JD in their deer at some stage which indicates the disease is very widespread. However, 52% of the respondents who had seen JD in their deer felt that it had emerged within the ten year period prior to 2010, 30% felt it had emerged in the ten years to 2000 and only 10% saw the first signs of JD in their deer before that. This is broadly consistent with a typical epidemic curve of disease transmission through a population.

Diagnostic comparison

Eight herds were involved in the study over a two year period and a total of 2,349 R2 hinds were sampled for the screening Paralisa™ test. The positive test rate in the herds ranged from 1.4% to 50%, with a median rate (and average rate overall) of about 8%. The results confirm that both Paralisa™ and PARACHEK®2 ELISA tests are effective at detecting animals that are high MAP shedders as determined by faecal qPCR and that the sensitivity of the two tests was tests was similar. Estimates of sensitivity of Paralisa™ were similar to those published by O'Brien *et al.* in 2013.

Case studies

Three properties from the study were identified for the development of case studies. These three had recognised issues with JD, with JDSLN (JD-suspect lesion) rates of >2% in their processed deer and/or clinical cases on-farm. In all cases, the carcasses from animals with JDSLN were 23–27% lighter than those without and the financial loss from JD through lighter carcasses and deaths was estimated at $3 to $9 per stock unit farmed.

The case studies note the success of management practices implemented on farm in response to test results. The culling of deer with a high Paralisa™ test result and management of suspect animals were effective at reducing the rate of new clinical cases on these farms. As a general rule, the amount of testing for JD on farm should reflect the severity of the issue, with the expectation being that the scale of testing will decline as disease levels decline.

The studies support our understanding that properties that trade stock are in a more vulnerable position than closed operations. It was recommended that properties that supply weaner deer should follow a strict test and cull policy to minimise the transmission of disease between herds and that finishing properties should be encouraged to establish the status of the herds from which they source stock to minimize the risk of introducing the disease onto their property.

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Fennessy, Peter; and Jopson, Neville; AbacusBio Limited and Norton, Solis; Johne's Management Limited. *Deer Industry Intervention Study; JDRC Milestone 5.3.3.* Diagnostic Comparison Report. Johne's Management Limited. October 2015

Research highlights

Prevalence, strains and transmission

Population based estimates of prevalence in 2012 indicated 60% of dairy cattle, 52% of deer, 79% of sheep and 44% of beef cattle in New Zealand are infected with MAP. Levels of clinical disease are much lower, and within herd incidence of clinical disease is very low (<1%).

There was strong evidence that MAP is transmitted between species when animals are co-grazed, increasing the likelihood of infection on multi-species farms.

Based on the VNTR/SSR typing system developed by AgResearch, there are at least 20 sub strains of Type C and eight sub strains of Type S MAP found in New Zealand dairy cattle, beef cattle, sheep and deer however only four of these sub-strains were responsible for ~89% of all MAP isolates found on survey farms.

Both dairy cattle and deer are usually infected with Type C strains of MAP, however there is a clearly different Type C sub-strain found in dairy cattle to that found in deer. Sheep are usually infected with Type S strains of MAP and beef cattle with both Type C and Type S. Unexpectedly 80% of isolates in beef were found to be the type S strain which is usually found in sheep.

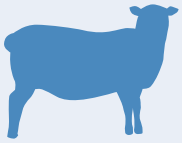
On some farms animals can be infected with more than one strain type of MAP, suggesting the animals have been infected on more than one occasion.

The incidence of JD shows regional variations in New Zealand, affecting deer and cattle most severely in the South Island and sheep in the North Island.

While increasing the risk of disease transmission, co-grazing appears to have potential beneficial effects on deer herds by reducing the incidence of clinical JD when jointly farmed with sheep.

Computer models have predicted that early detection and removal of high shedders will be the most effective means of reducing the impact of Johne's disease in a herd of deer or flock of sheep.

Surveys of deer, cattle and sheep have indicated that both infection with MAP and clinical disease affect productivity; lower pregnancy rates were seen in JD positive beef and deer herds, lower culling rates in JD positive beef herds, and higher culling rates in JD positive deer herds.



Sheep

Guidelines were published for the management of Johne's disease in sheep flocks.

Annual mortality due to OJD on farms suspected of having a high prevalence of JD was found to be between 0.7–2.8%, with higher mortality rates in fine wool breeds. Overall mortality rates in all breeds on these farms was between 7-8%.

A number of conditions (including include parasites, poor molars, poor legs or feet, enteric and respiratory disease) were shown to contribute to mortality on farms with a high OJD prevalence rate.

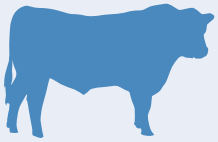
The annual cost of Ovine Johne's disease was estimated at $2.2–3.2 per adult ewe in 2015. With 75% of the 38 million adult sheep estimated to be affected with OJD, the costs at a national level were estimated to be in the order of $75–92 million.

Data collected on farm suggested that ewes with JD are culled or die at least six months earlier than their flock mates.

Modelling data predicted that vaccination would be cost effective in flocks where OJD mortality was ≥1.8%, but would not be cost effective below 1% mortality.

Modelling data suggested that vaccination could be expected to reduce ewe mortality in a flock from 2.75% to zero over nine years using continuous vaccination.

Computer modelling showed that co-grazing beef and sheep increased the prevalence of disease in both species. The longer the co-grazing period the higher the prevalence becomes in both species.



Beef

Clinical Johne's disease was found to be rare in beef cattle in New Zealand. Most herds were infected with Type S strains of MAP, likely due to direct contact between sheep flocks.

Computer modelling suggested that JD is more difficult to control in sheep than beef cattle as interventions, such as test and cull, reduce prevalence faster and to lower levels in beef than sheep.

Deer

ELISA and qPCR tests were shown to be useful in the control of JD and had a positive cost benefit ratio when used appropriately. The outcome of testing showed the best results when integrated with a whole herd health and farm management plan.

Paralisa® and Parachek® ELISA tests showed comparable performance across a range of shedding rates in deer. The Sensitivity of ELISA tests was shown to increase with increasing shedding rates (from ~73% at ≥103/g to 100% at ≥106/g faeces in the JDRC study).

Average annual loss rate due to JD on deer farms in 2014–15 was $1.33 per stock unit.

Trials proved that young deer were more likely to develop clinical disease on exposure to challenge with MAP than older animals.

Several key genes were found related to MAP infection in deer. Their function suggests that susceptible animals develop severe disease due to uncontrolled inflammation and cell death processes. Resistant animals appeared to be able to control cell death. A list of genes which potentially may be markers for signalling resistance or susceptibility to JD in deer was compiled.

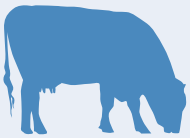
Genetic parameters for measuring Johne's susceptibility in deer were found to be moderate (0.16 to 0.26) and highly genetically correlated (0.85 to 0.94) in red deer. Heritability's were low in Wapiti.

Survey data indicated that farmers that used consultants and participated in discussion groups were more likely to recognise the true impact of JD than those who did not.

On-farm trials suggested that a proportion of deer lesions in the gut caused byJD could self-cure (i.e. lose visible signs of the infection).

Computer models in deer predicted that rotational grazing would be preferred for disease control in deer over permanent set stocking to minimise bacterial loads on pasture.

All of the common strain of MAP were found in lesions in the lymph nodes of deer at slaughter, indicating that these strains were all capable of causing clinical disease in deer.



Dairy cattle

Guidelines were published for the management of JD in Dairy herds, including advice on diagnosis in herds.

Bulk milk vat ELISA testing has been shown to be a useful tool for screening dairy herds for JD, but screening should not be attempted in late lactation as raised antibody levels in milk interfere with test performance.

Over 5000 dairy herds were screened for antibodies against JD by bulk vat milk ELISA in 2008-2010; 1% herds tested positive and 5% herds were classified as suspect for the presence of antibodies to the disease.

Dairy cattle have been shown to most likely to test positive to JD between lactations 3–6.

Results indicated there is limited value in testing for JD infection in young cattle as both culture and serology can fail to detect infected animals.

Results showed that clinical JD reduces the productivity of dairy cattle both seasonally and across the lifetime of a cow. Milk, fat and protein yields could be significantly lower in JD positive cows.

Survey data indicated that herds are more likely to see clinical JD on farm with increasing herd size and if replacement heifers are transported off farm at ≥5 months of age or calves are raised on the property of birth for at least one month post-weaning.

A DNA bank from ~2000 Johne's affected dairy cows was established and DNA genotyped to find genes related to resistance and susceptibility (R&S) to JD. A commercial test was developed for R&S and implemented for JD, but validation studies have indicated it may to be too costly to maintain.

A reliable challenge model for inducing MAP infection in dairy cattle was developed, which had been an area of difficulty for researchers worldwide.

Jersey cows were shown to be three times more susceptible to JD than Holstein-Friesians.

Studies demonstrated that in dairy cattle with severe JD, MAP bacteria survive in the gut because the immune system fails to recognise that MAP is a threat and does not respond as it should.

Simulation modelling suggested that JD may reduce income on a dairy farm by 6–15%. Grazing calves separately on pasture never grazed by adult cows and never sprayed with effluent from adult cows reduced JD effectively within 10–15 years and was the most profitable intervention. The model also indicates that investment into annual testing (ELISA, PCR) for identifying infected and/or shedding cows, while reducing JD, may not be cost-effective in the long term.

Industry engagement

Developing resources for farmers

When the JDRC was established in 2008, deer was the only sector which had any significant information published for the management and control of Johne's disease in New Zealand. The Deer Industry resources formed the basis for developing guidelines for the rest of the livestock sector in New Zealand.

The JDRC has worked with industry bodies and the JDRC research team to develop guidelines for the management of JD in sheep, dairy and beef cattle which incorporate findings from the JDRC research program. These guidelines have been published by the industry bodies and are available on-line from the following websites:

Information for dairy farmers
www.dairynz.co.nz/animal/health-conditions/johnes-disease

Information for sheep farmers
www.beeflambnz.com/Documents/Farm/johne-sheep.pdf

Information for beef farmers
www.beeflambnz.com/Documents/Farm/johne-beef.pdf

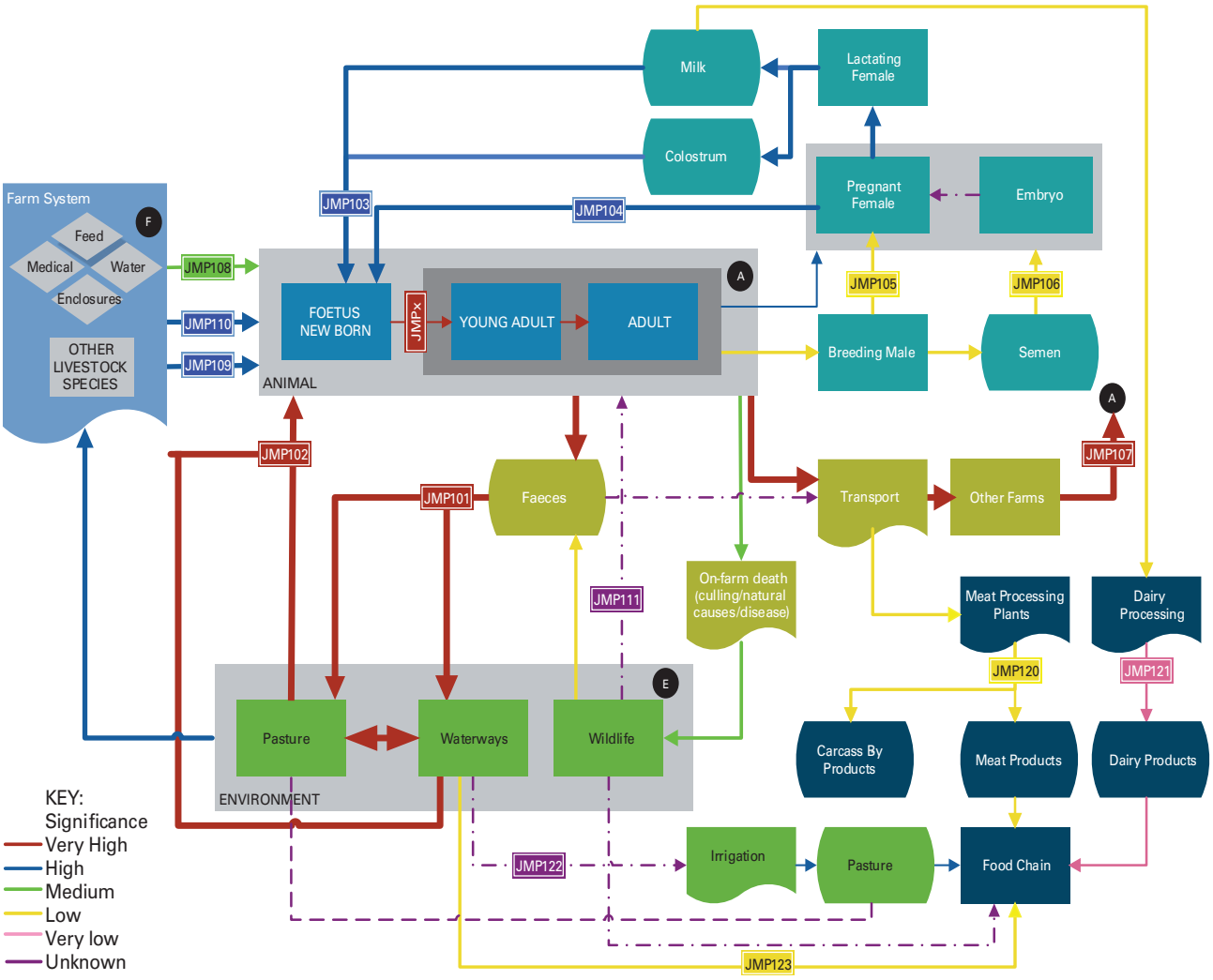
Information for deer farmers
(developed by the deer industry)
www.johnes.org.nz/publications

Disease control

The MAP transmission diagram was formulated by the JDRC in 2010 to describe the routes by which the bacteria move around a farm environment. The disease can be controlled by reducing the transmission of bacteria along these routes.

Such interventions to reduce transmission are described as Johne's Management Points (JMP's). The MAP transmission diagram has been used by industry to review controls in place to manage the disease and to develop practical interventions for New Zealand farming systems, on which the published guidelines are based.

MAP transmission diagram



Industry status and priorities for research

The primary driver for the JDRC research program has been the need to supply practical on-farm tools for industry to manage the impact of JD in herds and flocks in New Zealand. The JDRC has worked with its industry participants to formulate industry targets for the control of JD for New Zealand and has also regularly reviewed the status of industry management systems and priorities for research and development.

DairyNZ, Beef + Lamb New Zealand and Deer Industry New Zealand have adopted the following high level overarching goal for Johne's disease control and management in New Zealand, namely that: "Johne's disease does not effect on farm productivity and performance".

Each industry has identified activities that they are undertaking to achieve this target appropriate to the impact of JD on their sector. A paper commissioned by the JDRC in 2016 describes the status of JD control and management in New Zealand which is summarised in the table below.

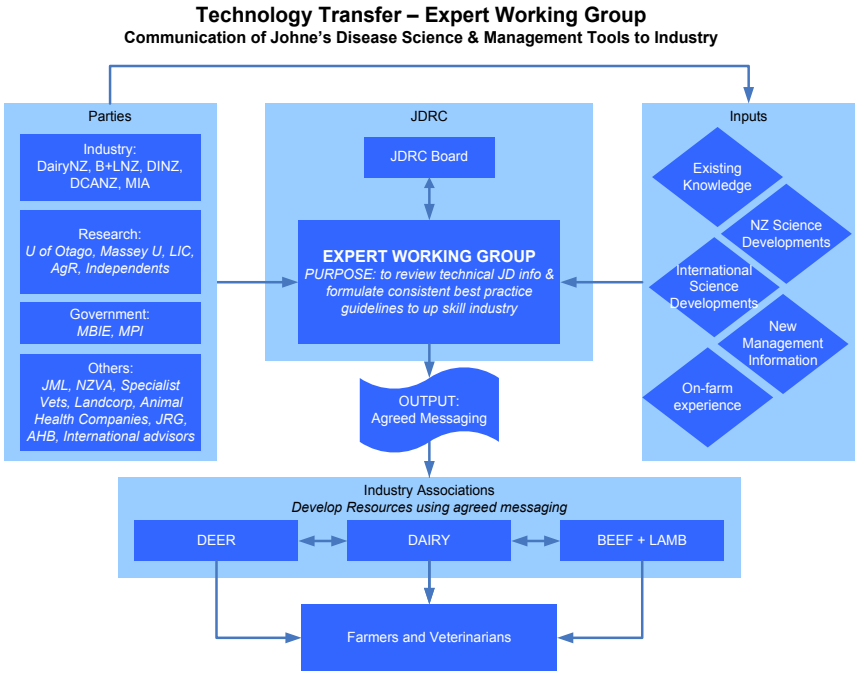
	Deer	Dairy	Sheep and beef
Aim:	Effective management of Johne's disease in deer	To reduce the exposure of calves to MAP and hence the prevalence of JD in the New Zealand dairy herd	To increase awareness of JD and provide management tools for high prevalence farms
Surveillance:	Slaughter house Monitoring of Stock overseen by JML	Not undertaken as means to accurately assess JD status is not available	Not undertaken as means to accurately assess JD status is not available
Resources:	JML publications Technical manual (2009) Farmer manual (2010) JML notification letters JCN Veterinary Consultancy Network and training support	DairyNZ Publications Dairy Toolbox (2015) Diagnostic Guide (2016) Vet Scholar Course (2014)	B+LNZ publications Sheep guidelines (2016) Beef guidelines (2016)

Johne's Advisory Group

The work of the JDRC has provided a solid platform to support industry in their ongoing need to ensure JD remains a low level threat to New Zealand livestock and to demonstrate internationally that New Zealand is aware and taking a coordinated approach to managing JD. This is particularly relevant should concerns regarding the zoonotic nature of the organism change and cause market access and safety issues.

In 2013 the JDRC established the Johne's Advisory Group (JAG), a cross-sector committee reporting to the JDRC Board for the purpose of providing expert review of technical information for New Zealand industry. The group is made up of individuals with JD expertise from across a number of differing disciplines and includes representatives from industry, academia and on-farm. The role of the JAG is to review JD technical information (including research outputs and management control measures from New Zealand and globally) and formulate consistent information and best practice guidelines to up skill industry. They also have a role in advising industry bodies regarding research priorities to improve the understanding of the disease in New Zealand.

With support from industry the JAG is to be retained as a cross sector forum for industry collaboration around JD control and management post the closure of the JDRC on 30 June 2016. The JAG will be responsible to monitor and maintain JD information and resources on an ongoing basis for the benefit of the New Zealand livestock industry.



TAG Membership (2016):

Solis Norton (JML)

Adrian Campbell (Veterinarian)

Peter Anderson (Veterinarian)

Rory O'Brien (Otago University)

Bob Jackman (MPI)

Roslyn Roberts (Fonterra)

Geoff DeLisle (ex AgResearch)

Hinrich Voges (LIC)

Geoff Ridley (B+LNZ)

Mandy Bell (JDRC, Veterinarian)

JDRC reviews

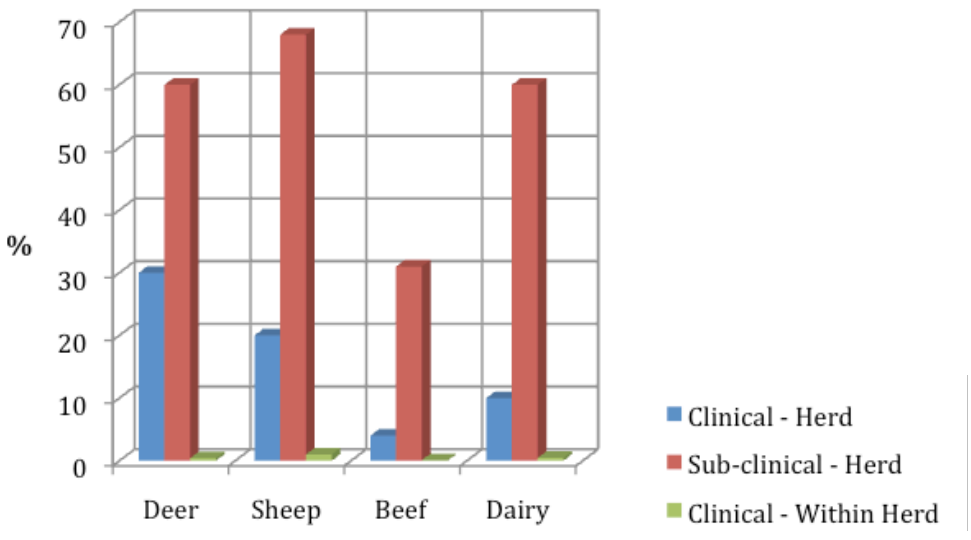
Alongside the main JDRC research programme the Consortium commissioned or authored a number of reviews to provide information to industry on issues of interest to the effective control and management of JD in New Zealand.

Prevalence of MAP in New Zealand

In 2011 the JDRC Board commissioned a review of the prevalence of MAP in New Zealand by Mark Bryan and Keryn Cresswell of VetSouth Limited. *"The Prevalence of Johne's Disease in New Zealand: A Review of Our Current Understanding"* was the first assessment of the impact of JD in New Zealand since the DeBrett review of 1998 and provided an overview of JD and its impact on New Zealand. The review assessed published records and interviewed key figures who had contributed to the understanding of JD in New Zealand.

The reviewers found that the nature of the disease and limitations surrounding diagnostics were such that establishing unequivocal prevalence data was a difficult task. The available data gave the best understanding of prevalence that could be made at the time, but was by no means complete. The general conclusions were that infection with MAP was widespread in New Zealand with >50% of all herds/flocks infected, but that the patterns found across a number of studies suggested that the rate of clinical JD was at fairly low levels in all species. In all species a significant tail of the population was seen where within herd prevalence (and incidence) was particularly high and within this part of the population there was likely to be significant economic loss and also the greatest risk of transmission of MAP in and between herds.

Prevalence of JD in New Zealand ruminants



Deer (2008-9)[1, 2, 3]		
Herd Prevalence	Clinical: ~30%	Subclinical: ~60%
Within Herd Prevalence	Clinical: ~0.4%	-
Sheep[4, 5, 6]		
Herd Prevalence	Clinical: ~20%	Subclinical: ~68%
Within Herd Prevalence	Clinical: 0.5-1.0%*	-
** Anecdotal data only*		
Beef cattle[7]		
Herd Prevalence	Clinical: ~4%	Subclinical: ~31%
Within Herd Prevalence	Clinical: <0.1%*	-
** Anecdotal data only*		
Dairy cattle[8, 9]		
Herd Prevalence	Clinical: ~10%	Subclinical: ~60%
Within Herd Prevalence	Clinical: ~0.45%	Subclinical: ~2%

[1] Heuer, C.; Wilson, P.; 2011. *JDRC Platform: Epidemiology and Herd Control Johne's Disease Research Consortium 2011 Review Evidence Portfolio.*JDRC Annual Science Review report Year 3 (July 2010 to June 2011)

[2] Stringer, L.A.; Wilson, P.R.; Heuer, C.; Mackintosh, C.G.; Glossop, J.C.; Verdugo, C.; 2009. *Isolation of* Mycobacterium avium *subsp.* paratuberculosis *from grossly normal mesenteric lymph nodes of slaughter deer, preliminary results from a nationwide prevalence study.* Proceedings of a Deer Course for Veterinarians, New Zealand. 2009 26, 67-71.

[3] Glossop, J.C.; Wilson, P., Heuer, C.; Castillo-Alcala, F.; Mackintosh, C.; 2008a. *Characterisation of clinical Johne's Disease on New Zealand deer farms.* Proceedings of a Deer Course for Veterinarians, New Zealand. 2008 25, 36-43.

[4] Gumbrell, R.C.; 1986. *The history and current status of ovine Johne's disease in New Zealand.* Proceedings of the 16th Annual Seminar, Society of Sheep & Beef Cattle Veterinarians NZVA, 1986, 173-184.

[5] Nuttall, 1991; Nuttall, W.; 1991. *The prevalence of Johne's disease*. Surveillance 18, 11-12.

[6] Staples, P.; 1994. *An update on Johne's disease in New Zealand.* Surveillance 21, 14-16.

[7] Staples, P.; 1994. *An update on Johne's disease in New Zealand.* Surveillance 21, 14-16.

[8] Voges, H.; 2008. *ONE bug, MANY cows, FEW dead: A descriptive analysis of Johne's culling data amongst New Zealand dairy cows from 1998/99 to 2006/07.* Proceedings of the combined meeting of the Food Safety, Animal Welfare & Biosecurity and Epidemiology & Animal Health Management Branches of the NZVA, 2008, 191-202.

[9] Ryan, T.; Campbell, D.; 2006. Mycobacterium paratuberculosis—*A Public Health Issue?*

Vaccination

While vaccination is known to an effective control measure to reduce faecal shedding of MAP and levels of clinical disease on farm in sheep, dairy cattle and deer the uptake of vaccination in New Zealand is currently low for all species. Vaccination is not favoured for a number of reasons including adverse injection site reactions, cross reactivity with TB testing and the down grading of vaccinated carcases at processing.

In 2014 the JDRC commissioned a review of vaccination as a control tool for the management of JD in New Zealand livestock by Terry Ryan of Ryan analysis.

The Consortium review was prompted by a number of developments to improve the landscape for vaccination, including improved vaccines, the introduction of identification and tracing of dairy cattle and deer to help identify vaccinated animals, the availability of tests to rule out cross reactivity issues and changes to post mortem inspection requirement at processing works. The aim of the review was to provide an assessment of vaccination as a control tool for JD in New Zealand considering the effectiveness of vaccination as a control tool and the identifiable impact and consequences of vaccine use on both animals and product.

The review found that there was a place for vaccination in sheep and in capital stock in deer and cattle in the JD control "tool box" especially where there was a high incidence of clinical disease. The two vaccines available in New Zealand (Gudair™ and Silirum™) were found to be both cost effective and effective at reducing production losses caused by MAP infection, even if not being totally protective against infection nor the establishment of shedding in a flock or herd. Vaccination, however, is not without problems, through operator associated self-inoculation and interference with diagnostic testing in deer and cattle; both for tuberculosis control and for control of JD via immunology test based management programs and these factors need to be considered if vaccination is taken up as a tool.

The review concluded that management of JD is very challenging and affected by many factors and that this difficulty supports, from an operational perspective, having multiple control tools and a set of well-informed advisors who can analyse individual situations existing in herds or on-farm and develop appropriate short to long term control measures. A program which integrates testing, management and vaccination could be the best option for control for some farms.

Lesions caused by MAP infection

The observation of JD like lesions in lymph nodes of farmed deer at slaughter is used as a surveillance tool by the deer industry to identify high risk JD herds in New Zealand by Johne's Management Limited (JML). In 2015 the JDRC noted a report of similar methods being used as a means of surveillance for JD in sheep flocks in Australia. Lesion surveillance has the potential to be a low cost means of monitoring the prevalence and therefore the JDRC undertook a preliminary review of the utility of using lesions associated with Johne's disease at slaughter as a diagnostic tool in sheep and cattle. Input from the review was obtained from literature and experts in New Zealand.

The information reviewed suggested that there was sufficient data to support the premise that visual inspection of the intestines and lymph nodes of animals at slaughter could be used as a means to monitor for (primarily) clinical JD in both sheep and cattle, as it does in deer. Animals in advanced stages of disease are highly likely to have macroscopic lesions that provide visible evidence of the disease at slaughter.

However, the review concluded that while lesion detection at slaughter might have utility as a low level surveillance tool, further information was needed to establish the frequency that lesions were seen in cattle or sheep at slaughter in New Zealand to determine usefulness of the technique. JDRC studies have suggested that very few sheep with visual signs of disease are sent for slaughter at a processing plant in New Zealand, and there is no data available regarding the frequency with which lesions are observed in cattle.

Johne's Disease Research Consortium Partners

Participants

AgResearch Ltd

Beef + Lamb New Zealand Ltd

DairyNZ Limited

DEEResearch Ltd

Livestock Improvement Corporation

Massey University

University of Otago

Associate members

Dairy Companies Association of New Zealand

Meat Industry Association Inc.

Research providers and contributors

Abacus Bio Limited

AgResearch (AgR)

Livestock Improvement Corporation (LIC)

Massey University

University of Otago

Research partners

Johne's Management Limited

Landcorp farming Ltd

New Zealand Merino Limited

New Zealand Government Funding Partner

The Ministry of Business, Innovation and Employment (MBIE)

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