A study of the biological and financial impact of OJD in affected sheep flocks in NSW

Mr Russell Bush
Research support: Meat and Livestock Australia.
Supervisors: Dr JA Toribio, Dr P Windsor, Dr S Webster

Debate continues regarding the impact on infected farms of Ovine Johne’s disease (OJD), a chronic enteric disease of sheep caused by the bacterium *Mycobacterium paratuberculosis*. Accurate estimation of annual mortality rates and the proportion attributable to OJD could provide an insight into the financial significance of this disease. The study quantified OJD mortalities in 12 flocks across four districts of south-eastern NSW, confirming considerable mortality rates (average 6.2%, range 2.1 to 17.5%) contributed to significant financial loss during the 12-month study period. Industry groups can now be provided with accurate figures on direct OJD losses within the endemic area of NSW. The data can be used to justify vaccination programs, and contribute to the development of cost effective strategies for future control and management. Further work on economic modelling will lead to tools being made available to farmers to enable them to optimise disease control programs.

Remote area syndrome surveillance systems for cattle

Mr Richard Shephard
Research support: Meat and Livestock Australia, Australian Biosecurity CRC
Supervisors: Dr JA Toribio, Dr Peter Thomson, Dr Angus Cameron, AusVet Animal Health Services

Remote extensive cattle grazing regions of Australia are characterised by large herds, long distances between properties and communities, little requirement for veterinary input, sparse veterinary services and inadequate surveillance coverage. This project investigates the potential for syndromic disease information to be captured by lay observers using the disease diagnostic program BOVID, analysed to compare prevalence of symptoms across regions and time periods, and used to determine the relative likelihood of individual disease occurrence. Changes in relative frequencies result in a ‘trigger’, notifying local government veterinary authorities that a given disease syndrome has emerged or changed in frequency, potentially leading to targeted surveillance efforts being focused towards investigation of the syndrome.

Identification of risk factors for OJD infection-level in sheep flocks

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Research support: University of Sydney International Postgraduate Research Scholarship, Meat and Livestock Australia.
Supervisors: Dr JA Toribio, Professor R Whittington

This project is designed to identify risk factors for the expression of Ovine Johne’s Disease (OJD) in 100 infected flocks through a cross sectional study of 3-4 year old sheep. OJD prevalence estimates will be based on pooled faecal culture. Information about OJD flock history, flock management practices and management of the 3-4 year old cohort will be collected by personal interviews. In addition, soil samples will be collected from the properties for analysis. Statistically significant associations between potential risk factors and OJD infection-level will be identified. The study is particularly focused on identifying risk factors that can be manipulated by farmers to improve on-farm control of OJD.
In vitro survival and dormancy of *Mycobacterium paratuberculosis*

Mr Sanjeev Gumber  
Research support: University of Sydney International Postgraduate Research Scholarship and Meat and Livestock Australia.  
Supervisors: Professor R Whittington, Dr D Taylor

*Mycobacterium paratuberculosis* causes Johne’s disease, an economically significant problem in ruminants in most countries. This organism survives for long periods on pasture and soil, and as the infection is acquired by ingestion, control is difficult. This in-vitro study monitored the survival of the organism (sheep strain) following exposure to different time and temperature combinations. It showed that temperature flux has a more detrimental effect on the survival of *M. paratuberculosis* than peak temperature. Dormancy was also observed in these experiments. Further work will lead to characterisation of gene and protein expression in the organism during growth and induction of dormancy in experimental models. This knowledge will inform our understanding about the survival of the organism in the environment and also in the host during the development of OJD.

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Ovine Johne’s disease – investigating mortality rates, disease transmission and control

Ms Helen McGregor  
Research support: Meat and Livestock Australia, NSW Stud Merino Breeders Association, CSL and proprietors of the study properties  
Supervisors: Associate Professor P Windsor, Professor R Whittington

OJD infection in Australian sheep flocks continues to cause significant losses due to mortality but losses in the sub-clinical phase of the disease have not been widely investigated under Australian conditions. Estimates to be made in this study of sub-clinical losses include reduction in wool growth and retardation in growth rates and their relationship with the development of clinical disease. This may provide insight into disease pathogenesis. Obtaining an accurate estimate of crude mortality rates and mortality risk in a flock and the proportion of the mortality attributable to OJD will establish the cost of the disease. Determining the contribution of pasture contamination rates and age of sheep, when first exposed to the disease, to incidence of disease in a flock, mortality rate attributable to OJD, the incubation period and the timing of diagnosis will lead to greater understanding of possible strategies for control and monitoring of diseased flocks. Documentation of the effects of whole flock vaccination on faecal excretion rates and mortalities in a high prevalence infected flock will enhance the knowledge and understanding of the benefits of a whole flock vaccination strategy including effects on adult excretion and cyclical pasture contamination.
Comparison of the S and C strains of *Mycobacterium paratuberculosis* at genome and proteome levels

Mr Ian Marsh  
Research support: Meat and Livestock Australia, NSW Department of Primary Industries  
Supervisor: Professor R Whittington

Johne’s disease (JD), a chronic and incurable disease affecting many ruminant species, is caused by *Mycobacterium avium* subsp. *paratuberculosis* (*M. a. paratuberculosis*). *M. a. paratuberculosis* strains can be divided into two groups known as sheep and cattle strains. The host range for the cattle strain is quite broad but the sheep strain primarily affects sheep. S and C strains have different cultural requirements. Little is known about the differences between S and C strains with respect to mechanisms of host specificity and pathogenicity. A greater understanding of these characteristics at the genome level would greatly assist in the control and management of JD both in Australia and abroad. In this study the genomes of S and C strains were compared using representational difference analysis, genome microarray and proteomic techniques. The differences observed thus far are greater than were previously suspected from existing restriction fragment length polymorphism analysis data, include major genetic deletions, and may be related to phenotype.

Genomic and phenotypic comparison of isolates of *Mycobacterium sp.* that contain IS900–like elements

Mr Martin McLoon  
Research support: Meat and Livestock Australia, NSW Department of Primary Industries  
Supervisor: Professor R Whittington

Ovine Johne's disease (OJD) is an important economic concern of Australian agriculture. The early diagnosis and implementation of control measures on properties with infected stock is the most effective way in preventing further spread of the disease. Presently a diagnosis of OJD relies on culture of the causal agent, *Mycobacterium paratuberculosis*, highlighting a mycobactin dependent phenotype in conjunction with PCR for the insertion sequence IS900. Detection of IS900 is included as a confirmatory test, and in some cases, the sole test because it has been shown to be unique to *M. paratuberculosis*. However, environmental mycobacterial isolates have been discovered which cause a cross reaction in the PCR for IS900 creating a false positive result and indicating the existence of IS900-like insertion sequences. To prevent false positive results a post PCR test was developed. To ensure the validity of this IS900 PCR-REA there is a need to characterise the mycobacterial isolates that possess an IS900-like insertion sequence at both a phenotypic and genotypic level, as well as the individual IS900-like insertion sequences. This analysis will allow a better understanding of the threat they pose to OJD diagnosis and their taxonomic position within the *Mycobacterium* genus.
Apoptosis in sheep with *Mycobacterium paratuberculosis* infection

Ms Sally Browne  
Research support: Meat and Livestock Australia  
Supervisors: Dr K de Silva, Associate Professor D Emery

Johne’s disease is a chronic wasting condition of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (Mptb). Clinical disease is seen only in adult animals. More knowledge regarding the pathogenesis of this disease, including apoptotic responses during the course of infection, is needed to allow advances towards management and control of Johne’s disease. The aim of this project is to develop assays for detection of apoptosis in sheep then define apoptotic responses in sheep with OJD. In initial experiments, Merino sheep aged 7 months were orally dosed with the organism and samples were taken of many tissues. At this very early stage in the development of Johne’s disease, there were no significant differences in the percentage of caspase positive cells when lymph node cells were incubated in the presence of medium alone or *Mptb* antigen. However, at day 6 with *Mptb* antigen, cells from gut lymph nodes in animals exposed to the highest dosage tended to have a higher percentage of caspase positive cells than the medium controls. Cells from peripheral lymph nodes incubated with *Mptb* for 6 days had higher apoptotic activity than the medium only controls, regardless of exposure to *Mptb*. These results suggest that detecting apoptosis in response to *Mptb* antigen *in vitro* in lymph node cells may be useful in identifying animals exposed to *Mptb*. Further studies are being carried out at later time points in the disease.

Aspects of the pathogenesis of *Mycobacterium paratuberculosis* infection in sheep

Ms Kate Goldsmith  
Research support: Meat and Livestock Australia  
Supervisors: Professor R Whittington, Dr D Begg, Dr K Bosward

Johne’s disease is characterised by a lengthy incubation period, measured in years. It is well known that cows with severe infections commonly shed the causative organism, *Mycobacterium paratuberculosis*, into their milk, and may also infect their unborn calf *in utero*. The organism can be isolated from extra-intestinal sites in such cases. There is a smaller body of evidence for this in sheep, but infection of tissues outside the gut is recognised in advanced cases in rams and ewes. The means by which the organism reaches sites outside the gut is uncertain, but may involve transfer via blood. The aim of this project is to identify extra-intestinal transfer of the organism during experimental and natural Johne’s disease infections in sheep.

Genetic and proteomic differentiation of stages of *Mycobacterium paratuberculosis* infection

Ms Ling Zhong  
Research support: Meat and Livestock Australia  
Supervisors: Professor R Whittington, Dr D Taylor, Dr L Di Fiore

Johne’s disease is a chronic disease affecting ruminants and other hosts. It is caused by *Mycobacterium paratuberculosis*. The events leading to clinical disease are complex and poorly understood but obvious signs of disease are seen only in adult animals. The genome sequence of the causative organism was recently published and this has triggered a dramatic interest in genomics and proteomics to elucidate mechanisms of disease. During this study a range of genomic and proteomic techniques will be applied to study the early stages of infection of sheep with this organism, and to contrast findings with those occurring later in the disease process. This information will be used to design improved diagnostic tests and disease control strategies.
Modelling the emergence of avian influenza in the Australian poultry industry

Mr Sam Hamilton
Research support: Australian Biosecurity CRC for Emerging Infectious Disease,
Department of Agriculture, Fisheries and Forestry
Supervisors: Dr Jenny-Ann Toribio, Dr Graeme Garner

Highly pathogenic avian influenza (HPAI) poses a serious threat to the Australian poultry industry and potentially also to public health in this country given recent occurrence of human deaths in south-east Asia. Disease modelling, by evaluating the behaviour of this disease under Australian conditions and the effect of alternate control strategies, is recognised as an important tool to support Australia’s preparedness for an incursion of this disease in our poultry population. This project, working with government and industry, will develop a new model of the spread of HPAI within the Australian intensive poultry population to address issues associated with assessing the extent, impact and control of disease outbreaks. This model will be used to enhance national disease planning and will provide technical underpinning for Australia’s outbreak management policy in the event of an outbreak of HPAI.

Peri-urban regional surveillance for biosecurity for pigs in eastern Australia

Mrs Nicole Schembri
Research scholarship: Australian Biosecurity CRC
Supervisors: Dr Trish Holyoake, Dr Jenny-Ann Toribio

Exotic diseases such as foot and mouth disease may enter Australia through anthropogenic means, most likely closely associated with large urban centres. Pigs in peri-urban settings may be the initial focus of a disease outbreak. This project involves reviewing current pig keeping legislation, educating producers about awareness of abnormal behaviours and clinical signs of disease, reviewing current extension materials and methods, as well as possible alternative animal identification technologies. The information obtained will be used to locate peri-urban pig producers, identify and track pig movements, and improve producer awareness of disease.

The sheep/ *Haemonchus contortus* relationship

Ms Kate McMaster
Research support: Meat and Livestock Australia.
Supervisors: Associate Professor N Sangster, Dr K Bosward

*Haemonchus contortus* is one of the most economically important parasites in the Australian sheep industry. In recent years resistance to parasitic drugs has forced both sheep producers and researchers to look for alternative means of parasite control. A better understanding of the host parasite relationship is an essential step in discovering improved control measures. The balance of the host parasite relationship shifts depending on the status of the host, for example age or reproductive status, and how the parasite responds. Studying the development of the protective immune response of the sheep when infected with *Haemonchus* will enable a better understanding of the host-parasite relationship and should provide the means to measure the development and mechanisms of immunity and resilience to infection. This will indicate whether we can predict or modulate the response towards protection or test the host’s “resistance status” for selection purposes. It is hoped that this investigation will provide producers with another tool to manage parasitic infections without resorting to anthelmintic treatment.
Specialised management of gilts and their progeny

Ms Yvette Miller  
Research support: Australian Pork Ltd  
Supervisor: Dr Trish Holyoake

The aim of the project is to reduce antibiotic use on pig farms to make the pork industry in Australia more globally competitive, and to benefit human health by reducing the risk of amplifying strains of antibiotic-resistant bacteria. The project seeks to: provide extensive training in pig health and production to provide for succession in the Australian pig industry; improve the pre-weaning growth performance of gilt progeny using supplemental milk; identify risk factors that explain why gilt progeny perform poorly, relative to sow progeny; and, develop management strategies to control the risk factors and hence improve their performance.