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## PHD RESEARCH STUDENTS

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

#### Mrs Kate Bower (nee Goldsmith)

Research support: Meat & Livestock Australia  
Supervisors: Professor R Whittington, Dr D Begg



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.

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### Apoptosis in sheep with *Mycobacterium paratuberculosis* infection

#### Ms Sally Browne

Research support: Meat & 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.

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### 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  
Completed: December 2005



Photograph: Kristen Clarke

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.

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

#### **Mr Navneet Kumar Dhand**

Research support: University of Sydney International Postgraduate Research Scholarship, Meat and Livestock Australia.

Supervisors: Dr J-A Toribio, Professor R Whittington

Completed: 2007



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.

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### **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

Completed: December 2006



*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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### **Immunopathology and development of diagnostic test for early detection of Johne's disease in cattle and sheep**

#### **Mr Ratna B Gurung**

Research support: Endeavour Postgraduate Award, 2009 (EPA 2009)

Supervisors: Professor R Whittington, Dr A Purdie, Dr D Begg



Johne's disease also known as paratuberculosis mainly in cattle and sheep is a chronic condition affecting intestines. It has worldwide distribution with varying prevalence in different countries. Dairy industries are badly affected in many developed countries. Due to the economic implications from loss of production it has become a concern for all. Although many diagnostic tests are available, they have their own limitations. Tests with low cost, as well as better sensitivity and specificity for early detection of disease are needed to improve the currently available diagnostic approach. Early detection of the disease will benefit farm profitability and welfare of subclinically affected animals.

In pursuit of identifying potential candidate antigen for use in diagnostics as well as vaccine development, this study will focus on various antigens from the causative agent genome. Detailed study of immunological values will be done on the identified antigens that will be used for future diagnostic tests for early detection of disease. This study is also prospecting collaboration between international institutions for some parts of the research component. The established research facility at the Faculty of Veterinary Science at Camden within the Johne's disease projects supported by MLA, will be the main research station for the entire study.

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## Detection of *Mycobacterium avium*. Subsp. *paratuberculosis* in sheep faeces by direct Quantitative PCR

### Ms Satoko Kawaji

Research support: Endeavour Japan Post Graduate Research Scholarship, University of Sydney, International Post Graduate Research Scholarship and Meat and Livestock Australia.

Supervisors: Professor R Whittington, Dr D Taylor

Completed: December 2009

Satoko's research is focusing on the detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in ovine faeces by direct quantitative PCR. MAP is a causative agent of Johne's Disease (JD), which is a chronic progressive enteritis of ruminants prevalent worldwide and causes significant economic damage to livestock farming. Although one of the gold standard tests to diagnose JD is isolation of MAP from shedding animals by culture, this organism grows very slowly and often takes several months to form a visible colony on solid media. To develop a new diagnostic method faster and more sensitive than culture, a real-time PCR based test is being validated for use on the detection of MAP DNA in ovine faeces. This tool will be used in basic pathogenic studies in sheep and cattle in the second part of the PhD program.



SKC Photography

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## 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

Completed: March 2006

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.



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

### Ms Helen McGregor

Research support: Meat & Livestock Australia, NSW Stud Merino Breeders Association, CSL and proprietors of the study properties

Supervisors: Professor P Windsor, Professor R Whittington

Completed: 2008

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.



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## 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

Completed: December 2007

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.



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**Immunogenic and pathogenic aspects of *Mycobacterium avium*. Subsp. paratuberculosis specific cell wall Lipopentapeptide**

**Ms Shyamala Thirunavukkarasu**

Research Support: University of Sydney World Scholars Award  
Supervisors: Professor R Whittington, Dr K de Silva, Dr K Plain



Paratuberculosis caused by *Mycobacterium avium* subsp. Paratuberculosis (*Map*) is one of the most important causes of morbidity in ruminants leading to huge economic losses worldwide. Control of mycobacterial infections requires the development of a Th1 type T cell response. The innate immune responses are believed to be triggered by pattern recognition receptors, such as toll like receptors (TLR). Several studies have shown that mycobacterial components act as TLR agonists. Lipopentapeptide is component found only in the cell wall of *Map* and is absent in the cell wall of other mycobacterial species, hence it is unique to *Map*. This project will study the immunogenicity of lipopentapeptide and the interaction of lipopentapeptide with TLR and the resultant impact it has on the pathogenicity of the organism. Studying this would be useful in understanding the pathogenesis of *Map* as well as to determine if this *Map* specific lipopentapeptide could have any putative diagnostic and therapeutic value.

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

**Mrs Ling Zhong**

Research support: Meat & Livestock Australia  
Supervisors: Professor R Whittington, Dr D Taylor, Dr L Di Fiore  
Completed: December 2008



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 were 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.

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**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.

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## Investigation of various aspects of ovine footrot control by specific vaccination

### Mrs Vidya Bhardwaj

Research support: Australian Wool Innovation  
Supervisors: Dr O Dhungyel, Professor R Whittington

A research team based at the University of Sydney has been established to achieve eradication of ovine footrot by specific vaccination. Ovine footrot is caused by many different serotypes of the bacteria *Dichelobacter nodosus*. Effective immunity is provided by vaccinating against 2 serotypes only at a time. To achieve eradication of disease, a flock is serotyped and a specific vaccination used to eradicate that serotype.



Currently, a small percentage of sheep in a flock fails to respond to the vaccination. Our studies will investigate if this failure to respond is due to the host immune status or due to the nature of the footrot vaccination. Footrot is manifested either as a benign form or a more virulent one in a herd. A study of the protein structure of virulent bacteria is expected to show significant differences from the benign strains. This can then serve as a diagnostic tool to predict the severity of an outbreak. Evaluations of various sample sizes will show the smallest number of animals that are required for sampling to give a statistically significant result.

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## Studies of the Epidemiology and Risk Factors Involved in the Pathogenesis of Acorn Calf Disease in Australia

### Mr Peter White

Research Support: Australian Post Graduate Award, Meat and Livestock Australia  
Supervisors: Dr J-A Toribio and Professor P Windsor  
Completed: March 2010

Acorn calf disease is a congenital chondrodystrophy that has recently caused significant losses to many producers in southern NSW and northern Victoria. Affected calves are born with a range of skeletal deformities and are often stillborn or die soon after birth. Australian outbreaks appear to be associated with years of drought. Similar outbreaks have occurred worldwide, but a cause has never been established.

The University of Sydney, in collaboration with state Departments of Primary Industry and the NSW Rural Lands Protections Boards, and with support from Meat and Livestock Australia, will conduct research into identifying the risk factors for Acorn Calf Disease. A survey of rural veterinarians will assist in establishing the distribution of the syndrome, which it is believed is under reported. A case-control study will be undertaken to identify the risk factors for the disease in Australia, with the aim of providing producers advice to reduce the occurrence of Acorn calves.



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## **Topical anaesthesia in livestock- reducing pain and stress and enhancing wound healing and production**

**Ms Sabrina Lomax**

Research support: Jean Walker Fellowship Fund (APA equivalent), Australian Research Council Linkage grant, Animal Ethics Pty Ltd, Bayer® Australia.  
Supervisors: Professor P Windsor, Professor P Wynn, Dr Meredith Sheil



Livestock husbandry procedures including mulesing, castration, tail-docking and dehorning cause considerable pain and suffering, yet are routinely conducted in Australia without the employment of anaesthesia or analgesia. While these procedures may be justifiable for animal health and management reasons, there is growing consumer concern, both nationally and internationally, regarding the infliction of pain on animals. This has the potential to significantly impact Australia's exports as consumers move to select alternative products or those from more welfare conscious markets. This could not be more evident than in the current mulesing debate that has put the Australian wool market under intense scrutiny by international importers. The growing pressure to select wool from un-mulesed sheep has led to an industry push to phase out the procedure by 2010 should a *viable solution* not be found.

Our research group aims to develop industry and consumer acceptable methods of pain management in livestock husbandry, as well as introduce novel methods of pain assessment in animals. We have been conducting studies over the past 2 years on the application of a spray-on topical anaesthetic formulation, Tri-Solfen®, to the wounds inflicted by mulesing, castration and tail-docking. We have assessed wound sensitivity using Von Frey Hair stimulation, measuring local involuntary reflexes and central cognition. These responses were graded using a numerical rating scale (NRS). Observation of behavioural responses to pain was also conducted, along with a novel technique to assess wound healing which involved digital photography and computerized surface area measurement.

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## **The Use of Topical Analgesia for Improving the Welfare of Livestock Following Invasive Husbandry Procedures**

**Ms Crystal Espinoza**

Supervisors: Professor P Windsor and Dr G Cronin

Animal husbandry procedures such as castration, tail-docking and dehorning are routinely performed on millions of animals worldwide each year. Such procedures are performed for varying reasons including animal health, for facilitating management and for the safety of farm workers. While the conduct of these surgical procedures are important in commercial animal production, the procedures cause pain and distress and are traditionally performed without anaesthesia or analgesia. The main limitation to currently available pain relief options is that they involve too much time, expense and labour and thus are not economically viable for commercial production.



An alternative solution may be the use of topical local analgesia in the form of a liquid substance containing local anaesthetics, which is applied during or immediately after a surgical procedure. Topical analgesia is more practical and affordable than current options and has the potential to be fast-acting and highly effective. Topical analgesia has recently been found to alleviate the pain associated with castration and tail-docking in lambs and calves, and mulesing in lambs. It is hypothesised that its application can be successfully expanded to surgical procedures applied to other production species. Findings from this research have the potential to improve the welfare of production animals, which has become a critical issue in modern society.

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**Reducing antibiotic usage in pig herds: controlling *Lawsonia intracellularis* by vaccination, housing and hygiene.**

**Ms Meg Donahoo**

Research Support: Australian Research Council Linkage Grant, NSW  
Department of Primary Industry, Boehringer Ingelheim  
Supervisors: Assoc Prof D Emery, Dr T Holyoake, Dr A Collins.  
Completed: 2009



Proliferative enteritis (PE) is an intestinal disease of pigs caused by the intracellular bacteria *Lawsonia intracellularis*. It is a major profit-limiting disease for pig producers globally and affected herds suffer from reduced growth, diarrhoea and increased mortalities. A new vaccine, Enterisol Ileitis® is now registered for commercial use in Australia as an alternative to antibiotics to control PE in pig herds. The immunology section of this project involves undertaking experiments focussed on improving the efficacy of the Enterisol Ileitis® vaccine under Australian pig management systems and to induce immunity to Australian field isolates of *L. intracellularis*. Approaches include determining the protective efficacy of the vaccine before weaning, identifying immune correlates associated with vaccine protection and determining an optimal medication-free window for vaccination.

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**Assessing pig movement and associated risk of Classical Swine Fever transmission in Eastern Indonesia**

**Miss Edwina Leslie**

Research Support: Australian Centre for International Agricultural Research (ACIAR), Pork CRC, Dinas Peternakan, Kupang (West Timor)  
Supervisor: Dr J-A Toribio  
Associate Supervisors: Professor M Ward and Dr M Geong



ACIAR has developed a project to improve understanding of livestock movement and the risk of spread of transboundary animal diseases. The component in which PhD student Edwina Leslie will be involved focuses on Classical Swine Fever (CSF) in Eastern Indonesia, specifically Nusa Tenggara Timor (NTT). Hog Cholera, as it is locally known, was first confirmed in NTT in 1998. Since its introduction, this virus has produced high mortality rates for the pig populations across most of this province. Pigs have a significant cultural role within this area and thus there has been a severe economic impact for local communities as a result of this disease. Formal movement of pigs will be investigated through interviews with pig sellers and pig buyers in markets. Informal movement of pigs will be investigated through village interviews with pig farmers. Risk assessment and social network analysis of their movement, in addition to identifying key pig management techniques, is expected to contribute to the development of government policy to minimise and control spread of CSF.

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**Specialised management of gilts and their progeny**

**Ms Yvette Miller**

Research support: Australian Pork Ltd  
Supervisors: Dr T Holyoake, Dr A Collins, Mr R Smits  
Completed: 2008



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 the performance of grower pigs.

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

### **Mrs Nicole Schembri**

Research support: Australian Biosecurity CRC for Emerging Infectious Disease  
Supervisors: Dr T Holyoake, Dr J-A Toribio  
Completed: 2009



Photograph: Kristen Clarke

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.

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## **Epidemiological investigations into the 2007 equine influenza outbreak in Australia**

### **Mr Simon Firestone**

Research Support: Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease, Rural Industries Research and Development Corp.  
Supervisors: Dr N Dhand, Dr J-A Toribio, Professor M Ward



This project is designed to investigate the characteristics of the 2007 equine influenza (EI) outbreak in Australia in order to improve our understanding of the factors contributing to the spread of the outbreak and contribute to preparedness for future incursions of animal diseases in Australia. The research team, based at the University of Sydney, includes partners from government (NSWI&I, QDPIF & DAFF) and industry (RIRDC).

A case-control study is underway, involving collection and analysis of data from 200 horse premises from highly affected regions of New South Wales. The aim is to investigate risk factors for the spread of EI onto premises, in particular non-compliance with biosecurity measures as recommended by animal health authorities at the time of the outbreak. This will be followed by an in-depth analysis of the features of the epidemic using spatial epidemiological approaches to compare the intensity of transmission of EI between several geographic regions, under different management conditions and operation types. The project also involves network analysis of data of horse movements during and after the outbreak. It is envisaged that the project will help refine guidelines to assist disease control authorities make informed decisions in the event of a future incursion of EI in Australia. Detailed analysis of the spread of EI may also yield critical insights into the ecology and transmission of influenza viruses in general in naïve populations.

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## **The 2007 outbreak of Equine Influenza in Australia- an investigation of policy implementation and horse owners' biosecurity behaviours.**

### **Ms Kathrin Schemann**

Research Support: Australian Postgraduate Award, Rural Industries Research & Development Corporation, NSW Department of Industry and Investment  
Supervisors: Dr N Dhand, Dr J-A Toribio, Dr M Taylor, Professor M Ward



In 2007 Australia experienced its first outbreak of Equine Influenza (EI), causing substantial financial and emotional hardship to horse owners and industry participants. The use of biosecurity measures represents a major means of control for highly infectious diseases. This project aims to investigate horse owners' biosecurity perceptions and the factors driving their biosecurity behaviour in light of the 2007 outbreak. The information gathered during this project will inform extension activities targeted at the horse industry to improve disease awareness and biosecurity practices.

In addition, the project will investigate the implementation of the EI AUSVETPLAN, the national policy document describing the Australian approach and proposed control measures to manage an outbreak of EI. We will investigate factors influencing policy implementation, such as epidemiological information available at the time of the outbreak, media coverage, logistics, economics, animal welfare and lobbying. Elucidation of these factors will inform disease control authorities on how to improve policy development and implementation for potential future outbreaks of exotic infectious diseases.

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## **Application of molecular diagnostic techniques to epidemiological investigations of Viral Nervous Necrosis (VNN) in barramundi aquaculture**

### **Mr Paul Hick**

Research support: Australian Post Graduate Award (Industry), Australian Research Council Linkage Grant, Northern Territory Government, Darwin Aquaculture Centre and Berrimah Veterinary Laboratory

Supervisors: Professor R Whittington, Dr D Taylor and Dr J Humphrey

Completed: March 2010

A research team based at the University of Sydney with industry partners in the Northern Territory has been established to investigate Viral Nervous Necrosis (VNN), a devastating disease of juvenile fish. Caused by a *Betanodavirus*, VNN has emerged globally in the previous 20 years in many situations where fish are produced in hatcheries. Within Australia, VNN has proved to be an impediment to the development of the barramundi aquaculture industry as a consequence of unpredictable supply of juvenile fish and concerns regarding the dissemination of the virus into wild fish populations.



Comparison of existing diagnostic techniques to new molecular technologies is expected to provide a refined set of tools for gathering epidemiological information about VNN. Investigation of disease pathogenesis will be facilitated by a series of infection trials. A survey of wild barramundi populations will determine disease prevalence based on the existing disease control zones within the Northern Territory whilst a survey of wild fish populations will establish disease prevalence in a variety of fish species.

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## **Iridoviruses of fish**

### **Ms Anneke Rimmer**

Research support: University of Sydney Postgraduate Scholarship in Iridoviruses in the Murray Darling Basin

Supervisors: Professor R Whittington, Dr J Becker

Viruses within the family *Iridoviridae* have emerged as a major threat to the aquaculture industry and native finfish of Australia. Recently there has been a growing concern regarding the introduction of exotic ornamental fish viruses, in particular dwarf gourami iridovirus (DGIV) to Australia via the transmission pathway of imported ornamental fish. Iridoviral diseases are known to be important in aquaculture, with DGIV having caused significant losses of farmed Murray cod fingerlings in Victoria in 2003. The range and effect of DGIV and other iridoviruses on wild populations of native fish in Australian waterways is largely unknown. Accordingly this research project aims to increase the knowledge of iridoviruses which may be present in Australia's Murray Darling river system through the development and evaluation of tools for studying iridoviruses of fish, determining the range and effect of iridoviruses in Australia and determining the properties of DGIV. The outcomes of this research will be beneficial to industry, inform policy and facilitate the necessary ongoing protection of the Australian aquatic environment through conservation management and assessing the quarantine risks associated with iridoviruses and imported ornamental fish.



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## **Iridoviruses of ornamental fish and implications for Australian marine species**

### **Mr Jeffrey Go**

Research Support: Australian Post Graduate Award and Vice Chancellor's Research Scholarship  
Supervisor: Professor R Whittington

Iridoviruses, particularly those of the genus *Megalocytiavirus*, have been associated with mass mortalities in a range of cultured finfish species throughout Asia. Additionally, this group of viruses have been reported from a range of fish species in the ornamental fish trade. In 2003, a *Megalocytiavirus* associated disease outbreak occurred in a Victorian Murray cod farm. This represented the first occurrence of this viral group in Australian aquaculture, although the outbreak was contained and the disease subsequently eradicated from the facility. The extremely close genetic relationship between the viral isolate involved in this outbreak, and *Megalocytiavirus* isolates from ornamental fish suggests that the ornamental fish trade was the most likely origin of this virus. Recent research also suggests that marine fish may be susceptible to infection with *Megalocytiavirus* isolates from the ornamental fish trade, which could have severe implications for Australian marine ecosystems and emerging Australian marine aquaculture industries.

A research project is currently underway to further characterise the nature of *Megalocytiavirus* infections and to test the susceptibility of a number of Australian marine finfish using material originating from the ornamental fish trade. The project will also aim to develop improved diagnostic tests for this group of viruses including in situ hybridisation, and serological tests such as enzyme linked immunosorbent assay.



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## **Healthy Dogs Healthy Communities - Approaches to knowledge sharing education to sustain dog health initiatives in remote Indigenous communities**

### **Ms Sophie Constable**

Research Support: Australian Postgraduate Award, Australian Research Council Linkage Grant  
Supervisors: Dr RJ Dixon, Dr RM Dixon

Dog poor is poor in many remote Indigenous communities compared to urban and rural averages. The Healthy Dogs Healthy Communities Program aims to explore current dog and community health as well as investigating the effect of dog health and dog health education programs.

This study aims to evaluate the use of education as a health strategy by identifying and analysing factors that lead to a successful culturally relevant, evidenced based, generative curriculum education program in 6 remote Indigenous communities. It is expected that the results of the project will enhance effectiveness and sustainability of future dog health and community health initiatives in remote Indigenous communities.



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## Investigating role of wildlife and wild canids in transmission of *Neospora Caninum*

### Ms Jessica King

Research support: Invasive Animal Cooperative Research Centre

Supervisors: Professor P Windsor, Dr J Ellis, Dr P Fleming and Dr D Jenkins

Completed: 2010

*Neospora caninum* is a cyst-forming coccidian protozoan parasite which was first described as causing fatal neurological disorders in dogs and has since emerged as a major cause of abortion in cattle worldwide. It is estimated to cost the Australian dairy and beef industries up to \$30 million per annum. Research in NSW has suggested there is a canine-bovine life cycle involved in bovine neosporosis, with many outbreaks associated with the proximity to bush land, where wild canids are at higher densities. However, data to support the hypothesis that wild canids are a source of infection to cattle is lacking and the possible role of foxes and other carnivores including native animals in bovine neosporosis needs investigation. It is also possible that a sylvatic cycle involving wild canids and wildlife similar to that of hydatidosis prevails in areas where *N caninum* is endemic, with some preliminary studies suggesting infection may be common in some native wildlife, such as possums.



SKC Photography

The aim of this study is to gather evidence to test the hypothesis that there is an association between prevalence of *N caninum* in wild canids and other wildlife, and that in cattle. Faecal, tissue and blood samples from target animals will be obtained from localities adjacent to cattle herds where reproductive problems due to *N caninum* have been identified, and examined by diagnostic tests including ELISA or agglutination tests, and PCR's, in habitats adjacent to farms where endemic bovine neosporosis has been diagnosed. These data will enable spatial epidemiological analysis of the prevalence and distribution of *N caninum* serovars in wild canid and wildlife populations, potentially enabling modeling of neosporosis risk and assisting management of reproductive failure in cattle.

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## Understanding and mitigating domestic pig and wildlife interactions

### Miss Hayley Pearson

Research support and funding: Australian Pork Limited

Research support: Invasive Animals CRC

Supervisors: Dr J-A Toribio, Dr S Lapidge

Wildlife has been a source of emergent disease for livestock, causing billions of dollars of economic impact. There are a number of diseases in domestic pigs that were sourced from wild animal hosts. As domestic pigs may come into contact with a range of wildlife species in Australia this project firstly aims to determine what animal species are most frequently observed in and around piggeries and are of concern to commercial piggery owners in Australia. This will be determined by responses to a postal questionnaire that will be distributed to all commercial piggery producers in Australia via Australian Pork Limited. Further aims of the project include determining whether there are interactions between domestic pigs and wildlife species, whether these wildlife species are carriers of diseases infectious to the pigs, and determining whether the wildlife are the possible source of infection in the pigs. It is expected that this project will significantly contribute to the understanding of how wildlife contribute to the disease load on pig farms. The specific outcomes are expected to be recommendations to producers and the industry as a whole on mitigating risk.



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## **Understanding the risks of Foot-and-mouth disease to Australia: modelling disease spread in feral and domestic animal populations**

**Mrs Katherine Negus**

Research support: University of Arizona

Supervisor: Professor M Ward



Our ability to detect and appropriately respond to emerging and exotic diseases is critical for Australia's biosecurity. However, there remain large gaps in our knowledge of the potential spread and areas of greatest risk for such disease occurrences, thus limiting our preparedness. Considerable effort, particularly recently, has been applied to modelling animal disease spread via livestock movements. However, the disease threat from wild animal (feral and native) and unfenced livestock populations that might form disease reservoirs has received comparatively little attention; such uncontrolled animal populations can represent a considerable proportion of the entire susceptible animal population for a variety of diseases. These populations can exacerbate outbreaks, form reservoirs of disease, and might allow novel diseases to become endemic.

Specific key outcomes – improve our understanding of the potential spread of FMD in Australia by using a spatial disease spread model within a geographic information system framework; and identify critical risk factors that predict the outcome of FMD incursions, in order to improve surveillance and control strategies. Research components – disease risks maps will be developed to illustrate criticality of locations to incursions of FMD by performing a semi-quantitative risk analysis; population surfaces for susceptible animal populations will be developed; a series of targeted simulations will be run to identify the possible spread from likely disease initiation points; those conditions (environmental and population) that can lead to larger outbreaks of disease will be characterized and the model used to evaluate different management strategies to mitigate disease spread.

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## **Framework for evaluating economics of the decision making processes related to the development of animal health emergency plans and policy in Australia**

**Ms Sarah-Jane Wilson**

Research support: Australian Government Department of Agriculture, Fisheries and Forestry

Supervisor: Professor M Ward



Literature will be reviewed, including the history of emergency animal diseases, the new livestock revolution, globalisation, and the drivers of animal health policy. Policy making in Australia related to emergency animal diseases (government and industry perspectives) will be explored, via a survey of current attitudes and approaches to decision making and policy development, focused on members of the CCEAD and peak industry body representatives. Follow up interviews with participants will then be undertaken. A gap analysis will be used to identify areas of commonality or divergence. Current methods used for decision making in policy development will also be considered. Examples in cost-benefit analysis, linear programming, partial equilibrium analysis, input-output models and social accounting matrices. Sociological assessments (political, moral and ethical) will also be considered. Event analysis will be used to understand what paradigms we have about what methods we currently use, reasons for a focus on technical frames of analysis, and the issue of cost recovery and emergency cost mitigation (versus compensation). Relevant AUSVETPLANS will be examined. The resulting framework(s) development will be validated using case studies on disease emergencies that represent a range of issues - such as actual versus theoretical incursions, industry type, trade, environmental concerns, zoonotic disease threats and consumer benefit. Case studies will be undertaken on foot-and-mouth disease, *Varroa*, equine influenza and Hendra virus.

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## **The Spatio-Temporal Epidemiology of Rabies in Bhutan**

### **Mr Tenzin**

Research support:

University of Sydney International Scholarship, Australia

Supervisors: Professor M Ward, Dr N Dhand



Rabies is endemic in southern Bhutan. Domestic dogs, especially strays, have been the main source of infection of domestic animals and humans. Rabies causes economic losses to farmers and the government in the form of loss of livestock and the cost associated with the prevention and control measures. Therefore, it is important to understand in detail the epidemiology of rabies in Bhutan. The following epidemiological aspects of rabies will be studied as part of the PhD research program:

- Geographical Information System and spatial analytical techniques will be used to describe and generate hypotheses for the spatio-temporal distribution of rabies occurrences in animals in Bhutan.
  - Molecular and phylogenetic analysis: rabies virus genotypes will be characterised by laboratory examination of suspected brain samples, gene sequencing and then phylogenetic analysis. The genetic characterization of rabies virus has not been previously studied in Bhutan.
  - Knowledge, attitudes and practice (KAP) survey: a cross-sectional study using a structured questionnaire survey will be carried out in one of the rabies endemic areas of Bhutan to determine the level of people's knowledge, attitudes and practices of rabies and rabies control activities including rabies vaccination, various aspects of dog ecology and the relationship between dogs and humans in the area.
  - The epidemiology of dog bite incidence in humans in Bhutan: there is a large number of stray dogs and dog bite (whether normal or suspected rabid) is common, especially in urban areas of Bhutan. A retrospective (hospital based post exposure treatment database) and prospective (cross sectional questionnaire survey) study will be completed to understand the epidemiology of dog bites in humans.
  - Risk assessment: currently rabies is endemic only in the southern part of Bhutan. We will estimate the chance of spreading the virus into the interior part of the country through risk-pathway analysis. The information generated from this study will help in designing a rabies prevention and control program for Bhutan.
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## **Modelling the emergence of avian influenza in the Australian poultry industry**

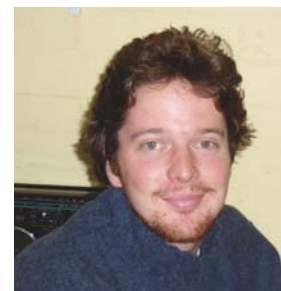
### **Mr Sam Hamilton**

Research support: Australian Biosecurity CRC for Emerging Infectious Disease,

Department of Agriculture, Fisheries and Forestry

Supervisors: Dr J-A Toribio, Dr G Garner

Completed: 2009



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.

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## **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

Completed: November 2006



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.

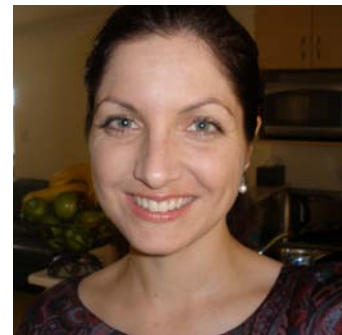
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## **Australian National Surveillance System for Animal Health (ANSSAH)**

### **Mrs Emma Fuller**

Research support: Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease

Supervisors: Dr N Dhand, Dr M Hernandez Jover, Dr A Cameron, Mr I Langstaff



The aim of Australian National Surveillance System for Animal Health (ANSSAH) project is to enhance Australia's capacity to provide timely information on animal health status. This will be achieved by drawing on innovative methodologies to develop a national system for the collection, storage, analysis and reporting of health information. The project will draw on the current National Animal Health Information System (NAHIS) and incorporate existing and potential data sources for surveillance that have varying degrees of utilisation. Stakeholders will be consulted about their specific requirements of surveillance information are what data is needed to meet those requirements. The ultimate outcome of the project is to implement a system that takes an active role in disease detection and surveillance data analysis and better positions Australia to address future animal health surveillance challenges in relation to livestock productivity, market access, public health, food safety, wildlife health and biodiversity.

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## **Cambodian field veterinary services and bovine health, with an emphasis on improved FMD control**

### **Mr John Stratton**

Research support: Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease, Australian Centre for International Agricultural Research

Supervisor: Professor Peter Windsor



The descriptive component comprises visiting Cambodian village animal health workers who are semi-skilled para-veterinarians responsible for delivering village veterinary services. Also involves analysing information on village animal health-worker training, their contact with farmers and the government, and their knowledge and experiences of the two most important diseases of Cambodian cattle and buffalo, haemorrhagic septicaemia and FMD, both of which are endemic by utilising village animal health workers to field trial FMD vaccination in the face of outbreaks.

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