Welcome to the Faculty of Veterinary Science’s Postgraduate Conference – 2011

Welcome to the Faculty of Veterinary Science annual postgraduate conference. The conference provides many opportunities for both our postgraduate students and our general research community. The conference is where our postgraduate students shine and inspire one another and the research community at large within the Faculty.

Importantly the conference provides a great opportunity for our postgraduate students to value add through developing professional and personal skills that will aid them as they become research leaders in their field. Enhancing communication and presentation skills, establishing networks and providing feedback are all outcomes that our postgraduate students traditional benefit from at this annual event. The audience (academic researchers, staff, undergraduates and fellow postgraduate students) will no doubt aid in the development of these skills through constructive feedback, ideas and acknowledgments in a positive and encouraging environment.

Producing a thesis and writing publishable papers are the main outputs for many of our postgraduate research students, this year’s conference activities will focus on the practical aspects of performing these integral tasks. We have lined up three speakers who'll provide some practical insights into utilising resources, academic honesty and scientific writing. From the Camden library, Poppy Prezios will give an informative and practical discussion on library resources and how best to use them. Dr. Megan Le Masurier from the Faculty of Arts, Department of Media and Communication will discuss the very important topic of academic honesty and final our very own Dr. Michelle Hyde will take our postgraduate students on the journey that is thesis writing.

This conference is made possible thanks to the generosity of our sponsors who we’d like to thank:

- Poultry Research Foundation
- Qiagen
- The Co-op bookshop
- Dairy Research Foundation
- Centre for Veterinary Education
- Meat and Livestock Australia
Finally, this conference has come together due to the hard work of Ms Marie Wildridge, supported by The Postgraduate Education and Research Training Committee (PERTC) and the postgraduate leadership group namely Ms Kathrin Schemann / Mr Sebastian Bowman / Ms Diana Jaramillo (Camden) and Ms YuanYuan Cheng (Sydney). I would personal like to complement the postgraduate student leadership group this year for their, communication, feedback and ideas on improving the postgraduate student experience at this Faculty.

I hope you all enjoy the 2011 postgraduate conference experience.

Dr Gary Muscatello
Sub-Dean Postgraduate Research
The Faculty of Veterinary Science
The University of Sydney
## CONTENTS

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABDELSAYED, MARY</td>
<td>8</td>
</tr>
<tr>
<td>ABDIRAHMAN, ALI</td>
<td>9</td>
</tr>
<tr>
<td>BARAL, RANDOLPH</td>
<td>10</td>
</tr>
<tr>
<td>BEGUM, FERDAUSI</td>
<td>11</td>
</tr>
<tr>
<td>BLACK, LISA</td>
<td>12</td>
</tr>
<tr>
<td>BOWMAN, SEBASTIAN</td>
<td>13</td>
</tr>
<tr>
<td>BRADBURY, EMMA</td>
<td>14</td>
</tr>
<tr>
<td>BROOKES, VICTORIA</td>
<td>15</td>
</tr>
<tr>
<td>BROWNING, LINDA</td>
<td>16</td>
</tr>
<tr>
<td>BYRON, ANNA</td>
<td>17</td>
</tr>
<tr>
<td>CAMPBELL, MICHAEL</td>
<td>18</td>
</tr>
<tr>
<td>CARR, MANDI</td>
<td>19</td>
</tr>
<tr>
<td>CHENG, YUANYUAN</td>
<td>20</td>
</tr>
<tr>
<td>CHONG, AMANDA YOON-YEE</td>
<td>21</td>
</tr>
<tr>
<td>CONSTABLE, SOPHIE</td>
<td>22</td>
</tr>
<tr>
<td>DURALI, TUGRUL</td>
<td>23</td>
</tr>
<tr>
<td>ESPINOZA, CRYSTAL</td>
<td>24</td>
</tr>
<tr>
<td>EVANS, NATASHIA</td>
<td>25</td>
</tr>
<tr>
<td>FIRESTONE, SIMON</td>
<td>26</td>
</tr>
<tr>
<td>FLETCHER, JESSICA</td>
<td>27</td>
</tr>
<tr>
<td>FOSTER, CHRISTIE</td>
<td>28</td>
</tr>
<tr>
<td>GO, JEFFERY</td>
<td>29</td>
</tr>
<tr>
<td>GOLDER, HELEN</td>
<td>30</td>
</tr>
<tr>
<td>GOMES-NOGUEIRA, MARIANA</td>
<td>31</td>
</tr>
<tr>
<td>GURR, JESSICA</td>
<td>32</td>
</tr>
<tr>
<td>GURUNG, RATNA</td>
<td>33</td>
</tr>
</tbody>
</table>
HARTIGAN, ASHLIE 34
HAWSON, LESLEY 35
HAWSON, LESLEY 36
HEYCOX, ELLEN 37
IQBAL, MUHAMMAD 38
JARAMILLO, DIANA 39
JARATLERDSIRI, WEERACHAI 40
KIMBLE, BENJAMIN 41
KOLBACH, RENE 42
LAU, QUINTIN 43
LESLEY, EDWINA 44
LILLIE, METTE 45
LIU, SONYA YUN 46
LOWE, JENNA 47
LYONS, NICHOLAS 48
MANSER, HARDY 49
MARCUS, ALAN 50
MAZRIER, HAMUTAL 51
MORRIS, KATRINA 52
MUSTIANA, ANA 53
NEGUS, KATHERINE 54
NG, JUSTIN 55
PEARSON, HAYLEY 56
PERDOMO, ALVARO 57
RANDHAWA, IMTIAZ 58
RANJBAR, SHAHAB 59
RAST, LUZIA 60
RICKARD, JESSICA 61
BREEDING FOR EXTENDED LACTATION IN AUSTRALIAN DAIRY CATTLE

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Second year, full time.

Introduction
Numerous changes in the dairy industry have taken place such as introduction of robotic milking and increased milk production which has led dairy producers to reassess if such traditional seasonal production systems are in fact the most optimal¹. An ongoing trend in the dairy industry is a shift to non-seasonal calving patterns providing scope to extending lactation beyond the traditional 305 days. Extending lactation has attested to increasing production and lactation efficiency through increased reproductive performance, decreased health issues associated with metabolic stresses around calving and early lactation, increased productive life of the cow, and increased profitability for the dairy producer². This project is aimed at examining the genetic and environmental variance components and obtaining estimated breeding values (EBVs) for extended lactation traits. In addition the project will assess the genetic covariance between extended lactation traits, persistency traits and other important milk and cow traits (survival, fertility indicator traits) which have not been estimated to date. Such findings will enable producers to select cows better suited to longer lactations and whether extended lactation traits should be in a breeding objective on its own or perhaps there needs to be a modification in the selection index to help producers maximise their profit from breeding.

Materials and Methods
Data obtained from ADHIS which includes ~158 million test day records from 1985 to 2010 from ~7 million cows and the extended lactation traits that will be looked at include milk yield, fat, protein, lactose percentage, Australian Selection Index (fat + protein-volume) and energy outflow of fat, protein and lactose as a measure of energy (MJ) per lactation. Extended lactation curves will be modelled using two methods, Wood model and random regression model to derive persistency and extended lactation traits that will be used in the genetic analysis. Genetic parameter, covariance estimates and estimated breeding values for these traits will be derived using linear mixed animal models using ASReml-R program.

Acknowledgements
Australian Dairy Herd Improvement Scheme (ADHIS), Melbourne
Dairy Futures CRC

References
WHOLE GENOME ASSOCIATION FOR LIVE UNTRAL SOUND SCANNED CARCASS TRAITS IN AUSTRALIAN BRAHMAN CATTLE

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Introduction
Genetic improvement for carcass and meat quality traits is an important component to the Australia’s beef industry, as both domestic and overseas consumers are placing greater emphasis on aspects of meat quality. The objectives of this study were to identify SNP (single nucleotide polymorphisms) associated with carcass traits using the genome-wide association study (GWAS) methodology.

Materials and Methods
A total of 565 Brahman heifers were ultrasound-scanned for eye muscle area (SEMA; cm²), rump fat thick (SP8; mm) and rib fat thickness (SRIB; mm) measured on two occasions: i) at the end of the first wet season, when the mean age of animals was 18 months and ii) at the end of second dry season, when the mean age of the animals was 24 months. These animals were also genotyped at 9,075 SNP markers using the Bovine 10k SNP Panel1. The phenotypes and genotypes were fitted in a mixed linear model as follows:

\[ \text{phenotype} = \beta_0 + \beta_1 \text{Age} + \beta_2 \text{SNP} + \text{MOB} + \text{Location} + \text{Farm} + \text{Animal} + \text{error} \]

using the ASReml-R software2.

Results and Discussion
Using a false discovery rate (FDR) cutoff of 10%, 10 SNPs were significantly (\(P \leq 1 \times 10^{-5}\)) associated with at least one of the phenotype considered. Five out of the 10 SNPs were on chromosome (Chr) 14 at genome location between 22 to 35 Mb for SP8 and SRIB, two SNP signals were detected on Chr 17 for SRIB and SEMA, where a single SNP signal was identified on Chr 5 for SRIB, Chr 6 and 8 for SP8.

Some of the associations found in this study are consistent with two recent published studies3,4. We also conducted genome-wide epitasis (SNP pair interaction) association analyses, and identified a set of SNP pairs that were significantly associated with carcass traits. Our study is the first to report genome-wide epitasis effecting on carcass traits in cattle. This study provides genomic tools for potential application of marker assisted selection (MAS) and a reference to assist with the identification of polymorphisms causing variation in ultrasound carcass traits in beef cattle.

Acknowledgements
The authors acknowledge Beef CRC for funding this project. Abdirahman Ali is a PhD student previously funded by Livestock Industries Division of CSIRO.

References
COMPARISON OF THREE IN-HOUSE BIOCHEMISTRY ANALYSERS TO A COMMERCIAL LABORATORY ANALYSER

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Introduction: In-house plasma or serum biochemistry analysis has become commonplace in veterinary practice. Several manufacturers produce and market analysers for this purpose and each manufacturer has in-house data that indicate accuracy when compared to commercial laboratory results. There is little independent data to confirm the accuracy of such tests. The utility of results from such equipment is entirely dependent on the accuracy of these results. Accuracy comprises precision and bias.

Materials and Methods: Commercially available quality control materials (QCM), Chemtrak® H-1 and Chemtrak® H-3 (Microgenics, USA) were used to assess within and between day precision of 13 analytes on Abaxis Vetscan® VS-2 Point of Care Analyser, Heska Dri-Chem®Veterinary Chemistry Analyser, Idexx VetTest® VT8008 and Idexx VetLyte®. The results are compared to those obtained by using the same QCM on a commercial machine, Hitachi-Roche, at a commercial laboratory. Bias of 13 analytes was assessed with 101 samples from 95 cats for which plasma biochemistry analysis was required for veterinary diagnostic purposes. Samples were collected by single jugular venipuncture and collected into a single lithium-heparin tubes (as defined by each manufacturer as being appropriate).

Results and Discussion: Precision: Assessed by Levene’s and associated tests showed approximately half of all in-house analytes (across all three machines and for both Chemtrak-1 and Chemtrak-3) assessed had comparable or better precision than that found at the commercial laboratory. Individual in-house analysers had comparable or better precision for 5-10 analytes. In many cases, precision results were comparable to the commercial laboratory results. Bias: Constant bias was seen to some degree for most analytes across all machines, this should be able to be overcome by adjustment of reference ranges. In most cases reference ranges have been adjusted appropriately by manufacturers. However, for 25/38 analytes (across all 3 in-house analysers) had notable proportional bias (ie amount of bias varied with concentration being measured).

Clinical Utility: Any effect that these precision and bias results have on clinical decision making is currently being assessed using three different metrics.

Acknowledgements: Gribbles Pathology (Heska Dri-chem and commercial laboratory), IDEXX Pathology, REM Systems (Vetscan) for providing analysers, analytes and allowing independent scrutiny of their products.
EFFECT OF YERBA MATE (*Ilex Paraguariensis*) SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY IN DAIRY COWS: IN SACCO AND IN VITRO STUDIES

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First Year full time (Master of Science in Veterinary Science)

Introduction: The use of herbs as additives in livestock nutrition, as an alternative to other chemical compounds, is becoming a new goal in livestock production. Studies in sheep² and dairy cows¹ suggest that Yerba Mate could be recommended as a natural novel feed supplement with the potential for improving feed intake and wool growth in lambs and to sustain milk yield in dairy cows.

Objective: The overall objective is to evaluate the potential use of Yerba Mate as a feed supplement for dairy cows.

The specific aim of this study is to evaluate the effect of Yerba Mate supplementation on rumen fermentation and nutrient digestibility (rumen degradability characteristics) of several feedstuffs (pasture, silage, concentrate) in dairy cows.

Hypothesis: Yerba Mate supplementation will improve pasture, silage and concentrate digestibility rate.

Materials and Methods: Three rumen-fistulated Holstein Friesian (age-3-4 years, weight 500-600 kg) cows will be separated from the rest of the herd and held in a small paddock (small grazing area close to the dairy). Cows will be fed Yerba Mate pellets (500g/cow/day) two weeks prior to the beginning of experiment.

In vitro incubations will be conducted to determine the products of degradation (ammonia and volatile fatty acids), whereas the in sacco technique will be conducted to determine the rate at which dry matter (DM) and its constituent chemical fractions are degraded through microbial digestion. The degradation characteristics of feedstuff (pasture, silage, concentrate) will be measured by incubating the samples in nylon bags into the rumen after morning meals and retrieve duplicate after 2, 6, 12, 24, 48 and 72 hrs. The bags will be put into cold water to stop ruminal bacterial activity and be washed with slow running tape water for 30 min. To determine washing loss, zero time bags (not incubated in the rumen) of each sample will undergo the same procedures as the incubated bags.

The disappearance of DM from nylon bags are fitted into the exponential equation $P = a + b (1 - e^{-ct})$

Where, $p$: the DM degradation after time $t$, $a$: rapidly soluble fraction, $b$: potentially degradable fraction, $a + b$: the potential degradation, $c$: the degradation rate of fraction $b$

For the in vitro gas technique, the samples will be incubated for 2, 6, 12, 24, 48 and 72 hrs, using rumen inoculum of the cows used for nylon bags.

Samples will be dried for 48 hrs in a forced air oven at 60°C and then weighed and ground to pass 1 mm screen and will analyse ash, crude protein, neutral detergent fibre, acid detergent fibre, water soluble carbohydrates.

Total nitrogen will be determined by Dumas Method (Block Digestion). Ash will be determined according to the AOAC, (1990). Neutral- detergent fibre (NDF), acid-detergent fibre (ADF) will be determined by the Refluxing method³. Water soluble carbohydrates will be determined by Water Extraction –Alkaline Anthrone.

The findings of this study may have the potential to be applicable to dairy industries and will give useful information to improve the management of dairy cattle.

References:


AN INVESTIGATION INTO THE PHARMACOKINETICS OF CHLORAMPHENICOL IN THE KOALA (Phascolarctos cinereus)

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Second year, full time.

Chlamydiosis, caused by Chlamydia pecorum and Chlamydia pneumoniae, is the most common infectious disease of koalas; while highly debilitating to the individual, it is also detrimental to the population as a whole. Chlamydiosis causes a syndrome of ocular and urogenital disease, with the latter frequently resulting in infertility. Chloramphenicol is one of the few antimicrobials available for treating chlamydiosis in koalas, as the traditional anti-chlamydial drugs have been shown to induce a fatal syndrome of inappetance, wasting and death due to their effect on the koala’s gastrointestinal flora [1, 2]. The current study was intended to characterise the pharmacokinetic profile of chloramphenicol following intravenous and subcutaneous administration to healthy koalas; the data from this study should enable the determination of a koala-specific dosing regime for chloramphenicol. 18 healthy koalas of both genders and various ages were administered chloramphenicol, either subcutaneously (n=12) or intravenously (n=6), and serial blood samples were taken for up to 48 hours post drug administration. The plasma portion of the sample was subjected to solid phase extraction followed by high performance liquid chromatography, to enable quantification of chloramphenicol in the plasma at each sampling time. Non-compartmental pharmacokinetic analysis was undertaken on the raw data, to yield a set of pharmacokinetic parameters for chloramphenicol in koalas.

The results indicate that whilst the base formulation of chloramphenicol has pharmacokinetic properties that make it suitable for use in this species, the plasma levels attained with the currently used dosing schedule are lower than the theoretical target plasma concentrations. In order to achieve the theoretical plasma target concentration of 5µg/mL, it has been calculated that the dose of chloramphenicol needs to be increased significantly, and administered to koalas twice daily. The potential for toxic side effects at this newly calculated dose is high, and is yet to be investigated.

ACKNOWLEDGMENTS
The Hermon Slade Foundation and The Australia Zoo Wildlife Hospital.

REFERENCES
BOVINE MAMMARY STEM CELLS AND THE LACTATION CYCLE

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Third year of full time PhD Candidature.

The milk production capacity of a dairy animal is influenced by the proliferation of mammary stem cells in the bovine mammary gland (Capuco et al., 2009). Despite the economic importance of this cell population little is known about bovine mammary stem cells, although comparable cell populations in other species have been characterised (Tiede and Kang, 2011). This project aims to understand the role of bovine mammary stem cells in the lactation cycle. Developing this understanding of bovine mammary stem cells may provide insight into opportunities to manipulate mammary cellular populations to prolong the lactation of dairy cattle at an economically viable level thus avoiding the need for a concurrent pregnancy to continue lactation. It also offers a route for genetic manipulation as a long term strategy.

Histological identification of bovine mammary cells
Mammary tissue biopsies from five Holstein Friesian cows were sampled at four stages of lactation. Samples were cryosectioned (10µm), and fixed in acetone and were co-incubated with fluorescently labelled antibodies to CD24 (Heat Stable Antigen), CD29 (Integrin β1) and CD49f (Integrin α6), a combination of cell surface markers identified in other species as indicative of mammary stem cells (Tiede and Kang, 2011) and counterstained with DAPI (nuclei stain). Using Imaris image analysis software the number of cells per section as well as the number of positive cells for each antibody was determined. The average numbers of cells positive for CD29 were highest during late pregnancy, falling by the time of colostrum production, increasing at peak lactation and dropping again at involution. The number of cells positive for CD49f followed a similar pattern, with a much greater decline during involution. Cells positive for these markers are located basolaterally to differentiated mammary epithelial cells in secretory alveoli.

Stem cells in bovine milk
Investigation of the cellular constituents of milk as a potential source of stem cells has also progressed as there may be a role for maternal cell micro-chimerism in neonatal development. Cells positive for the lineage-specific protein Nestin (neurological stem/progenitor cell marker) have been indentified in human breast milk (Cregan et al., 2007). This phenomenon has been explored in cell populations isolated from bovine milk. This was followed by co-incubation with labelled antibodies to Nestin, CD45 (marker of hematopoietic stem cells), Ki-67 (proliferation marker) CD29 and Sca-1 (stem cell antigen-1). The cells were analysed by flow cytometry with the addition of 7-AAD to ascertain membrane integrity as an indicator of viability. Positive subpopulations of Sca-1+ (9.7%), CD29+ (5.8%) or CD45+ (20.3%) were identified with few Ki-67+ cells observed.

Isolation of bovine mammary stem cells

A further aspect of this project is the isolation of putative mammary stem cells for in vitro culture. A strategy has been developed whereby a population of bovine mammary epithelial cells (BMECs) are enriched as CD49f positive through the use of immunomagnetic bead separation columns. Following this, antibodies to CD24 and CD29 will be utilised in conjunction with FACS to yield the subpopulation CD24+CD29+CD49f+ that is associated with mammary stem/progenitor cells in other species. This subpopulation will be cultured in order to assess the functional stem cell aspects, such as differentiative potential. The expression profile of this population will also be assessed using RNAseq technology.

References

Acknowledgements
The authors would like to acknowledge and thank Dairy Australia and the Faculty of Veterinary Science at the University of Sydney for funding this project.
EFFECTS OF DIETARY CALCIUM SOURCE ON BROILER LEG HEALTH

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First Year, full time

The modern broiler chicken has been genetically selected for rapid growth, increased muscle mass and heavier breast weight¹. This increase in production may be associated with poor leg health and lameness and is often linked to skeletal abnormalities due to reduced bone mineralisation (calcium and phosphorus deposition). Poor leg health and lameness affects millions of broiler chickens worldwide, with lame birds having significantly reduced performance and altered behavioural patterns²,³. As a consequence, poor leg health and lameness are important welfare concerns. This research aims to assess the efficacy of a more digestible source of dietary calcium on skeletal integrity and subsequent bird behaviour.

To investigate the effect of dietary calcium source on bird behaviour and skeletal integrity, 1820 Cobb-500 day-old chicks were allocated at random to one of 13 dietary treatments across 91 pens (7 replicates of 20 chicks). At days 26 and 42 of age, four focal birds from each pen were individually tested for skeletal weakness using the Latency to Lie test as described by Berg & Sanotra (2003). Other studies have shown that the behavioural response of broilers in the Latency to Lie test was highly correlated with gait scores, which is a widely used but subjective method for assessing lameness in broilers².

Latency to Lie results were significantly correlated (P<0.001) to body weight. Overall for every 100 gram increase in body weight the chance of the bird sitting in the water (lie) increased by 12%. There is a weak (P=0.06) effect of diet on the proportion of birds to lie. Further statistical analysis of the effect of individual diets to control birds found diets C and D, both different calcium sources at different levels, increased the time the birds remained standing. Birds fed diets C and D were found to be 59% and 54%, less likely to lie at any given point during the test, respectively. The results of the present experiment indicate that birds fed diet C and D were able to remain standing for longer periods of time, with fewer signs of clinical lameness. The finding that body weight is the main contributing factor to lameness is consistent with other studies⁴. Importantly, however, the present data shows that the dietary source of calcium may help improve skeletal integrity and reduce the clinical signs of lameness.

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MULTI-CRITERIA DECISION ANALYSIS TO PRIORITISE EXOTIC DISEASE RISKS TO THE AUSTRALIAN PIG INDUSTRY

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First Year PhD research (full time)

Australia’s domestic pig industry is geographically isolated and is free from more than thirty diseases that affect pigs overseas. The overall outcome of our project is to provide the Australian pig industry with information to aid in planning surveillance activities and response strategies for exotic diseases of most potential importance, using quantitative risk analysis and spatial disease modeling.

Defining “most important” in order to decide which diseases to investigate further is problematic. We can prioritise diseases on the amount of impact caused if a disease incursion should occur, but these pathogens vary in the way that they cause impact. The importance of different aspects of impact varies depending on the risk perceptions of the stakeholder.

We require a method of prioritising diseases that combines measures of impacts and the level of importance of impacts relevant to different stakeholder groups representing government and the pig industry. Over the last decade, semi-quantitative methods have been used for this purpose.

We plan to use two recognised quantitative multi-criteria decision analysis techniques; point of truth calibration (POTCal) using logistic regression[1], and probabilistic inversion (PI)[2]. The advantage of these methods is that they ask stakeholders to consider disease scenarios directly rather than make decisions about values of abstract disease attributes, they are repeatable, and can be validated. PI has been previously used in disease prioritisation[3], but POTCal has not. By using both techniques we can compare the methods in a disease prioritisation context, determining the most appropriate method to be applied in our study.

The stakeholder groups will be able to use the results of the prioritisation to direct their decision about which diseases should be investigated in more detail.

TO EVALUATE VITAMIN D3, CALCIUM, PHOSPHORUS, AND PHYTASE ON BROILER PERFORMANCE AND LEG HEALTH.
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First Year, Full Time, Master of Science in Veterinary Science.

Introduction: Leg health is an ongoing challenge for the broiler industry in Australia. The issues centre on the appropriate delivery of key minerals (notably calcium and phosphorus) in synchrony and at appropriate ratios for optimum bone health and maximum broiler production effectiveness. In summary leg health is dependent on a unique interaction of calcium, phosphorus and vitamin D3.

It has been demonstrated that a reduced calcium level in a diet can improve the effectiveness of exogenous phytase enzyme. However lower calcium levels in a diet may reduce and marginalize skeletal integrity. Higher levels of Vitamin D3 in reduced calcium diets supplemented with an exogenous phytase, may help maintain skeletal function¹. Furthermore, vitamin D3 has been shown to improve phytate phosphorus digestibility with and without exogenous phytase² ³. Higher intakes of Vitamin D3 may improve calcium and phytate phosphorus digestibility and improve leg health⁴.

Materials and Methods: In a 2x2x2 factorial feeding experiment, 288 male broiler chickens were fed for 28 days various levels of calcium, phosphorus, vitamin D3 and phytase in 8 treatment groups with 6 replicate pens of 6 chickens. Body weight, feed intake, calcium and phosphorus digestibility, bone ash and leg health were measured.

Results and Discussion: Between treatment groups there was no significant improvement in body weight. However, there was a significant improvement in feed conversion ratio between phytase inclusion and levels of calcium and phosphorus. Such an improvement is a major economic advantage. Statistical analysis of calcium and phosphorus digestibility and leg health is still to be undertaken.

Acknowledgements: This research was funded by the Poultry CRC of Australia.

References:
DETERMINING THE VALIDITY OF THE OVERALL HAEMOSTASIS POTENTIAL ASSAY IN DETECTING HYPERCOAGULABLE STATES IN DOGS.

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Second year, Part time.

Abstract
Global haemostasis assays such as thromboelastography and the platelet function analyser are gaining popularity in veterinary medicine¹. Whilst these tests provide information on the speed and extent of coagulation in animals, their application is limited by cost and the requirement for specialised equipment. The overall haemostasis potential (OHP) is a cost-effective global haemostasis assay for human samples, designed for use with standard plate readers² & ³. The aim of this study was to optimise the OHP for use with canine samples. Citrated plasma was collected from 60 clinically healthy dogs. The OHP assay and standard coagulation assays (prothrombin time, activated partial thromboplastin time, Fibrinogen, Factor VIII and von Willebrands factor) were performed for each animal. Pooled canine plasma was used for calculation of intra and inter-assay variation. Modifications to the assay were required. Canine plasma has a much shorter delay to clot formation than human plasma, therefore 90% less clot activator (thrombin) is required. Fibrinolysis (clot breakdown) is reduced compared with human samples, and the amount of tPA (fibrinolysis activator) required was increased by 16%. Using these modifications, inter- and intra-assay variation were <15% for most parameters. Preliminary results indicate that this assay will be suitable for detecting and monitoring increased risk of clotting (eg. stroke) in dogs. The validated OHP provides an inexpensive, accessible alternative to the other global haemostasis assays mentioned above. Its application in monitoring anticoagulant therapy and detection of hypercoagulability in dogs is being investigated.

Acknowledgements
R Churcher & J Braddock, North Shore Veterinary Specialist Centre, Crows Nest.

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OPPORTUNITIES FOR PASTURE BASED DAIRY FARM IN NORTHERN VICTORIA

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Second year, full time.

Introduction
This paper explores the use of a forage plan on a case study dairy farm located in Northern Victoria. The case study farm is one of four farms that are participating in a monitoring project being conducted through Future Dairy.

Materials and Methods
The case study farm is located near Katunga with 450mm annual rainfall with winter dominance. It is owner operated with one full-time staff member who assists with all operations. The farm has 110ha of milking area and currently milks 250 cows at peak with 60% calving in Autumn and 40% in spring. The irrigation water allocation is totals 689ML. The feedbase of the farm consists of 90% ryegrass pasture (both annual and perennial) and 10% lucerne. The surplus pasture in spring is conserved as silage and hay and fed back to the milking herd from January to early April.

Milk production is averaging 5000L/cow with 250kg butterfat/cow.

The farmer has the goal to maintain a pasture based dairy while increasing to 320 milking cows. The farmer also does not want to increase the amount of grain being fed per cow.

Two feed plans were developed to investigate replacing some concentrate with maize silage and also to increase milking cow numbers.

Results and Discussion

Table 1. Comparison of base year, using maize silage and increasing to 320 cows.

<table>
<thead>
<tr>
<th></th>
<th>Base Year 250 Cows</th>
<th>Base Year + Maize Silage</th>
<th>320 Cows + Maize Silage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoFC ($/ha)</td>
<td>$3653</td>
<td>$3754</td>
<td>$4174</td>
</tr>
<tr>
<td>Total Irrigation Water (ML)</td>
<td>439</td>
<td>519</td>
<td>535</td>
</tr>
<tr>
<td>Total Litres of Milk</td>
<td>1,243,543</td>
<td>1,319,940</td>
<td>1,609,502</td>
</tr>
<tr>
<td>Litres of milk/ML water (l/ML)</td>
<td>2832</td>
<td>2543</td>
<td>3008</td>
</tr>
<tr>
<td>Supplementary Feed Cost</td>
<td>$96027</td>
<td>$115,049</td>
<td>$182,408</td>
</tr>
</tbody>
</table>

The results of the three forage plans show that it would be possible for the farm to increase to milking 320 cows on the same area of land with a change in the forages grown and without increasing the amount of concentrate fed per cow.

Acknowledgements
This work was supported by Dairy Australia through the funding of Future Dairy and a PhD scholarship.
GASTROINTESTINAL PARASITE CONTROL MEASURES IN USE WITHIN THE CENTRAL TABLELANDS OF NEW SOUTH WALES

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2nd year Masters of Veterinary Science – Research part-time

Introduction:
Gastrointestinal parasites can cause major growth and productivity losses in young beef cattle. While recommended drenching programs have been developed for use in beef herds in the Central Tablelands of New South Wales, the adoption of these programs by cattle producers is largely unknown. As part of a larger study into the efficacy of worm control strategies focussing on Ostertagia ostertagi over summer months and the expression of Type II Ostertagiasis in autumn, a survey was conducted to determine what gastrointestinal parasite control measures are in current use by producers within the Central Tablelands, including the anthelmintics used in these control programs.

Method:
A questionnaire survey of 267 producers with greater than 250 cattle was conducted. Information on current drenching programs, anthelmintics used and the delivery method was collected and analysed.

Results:
The survey response rate was 19.5% (52/267). In 2009, 88% of producers elected to give an anthelmintic treatment at weaning. Weaning occurred between February and May in spring calving herds, with 73% weaning in the March-April period (16/41 weaning in March and 14/41 weaning in April). The most dominant anthelmintic group used was the macrocyclic lactones (used by 80% of producers) and over 60% of producers preferred a pour-on preparation. The survey identified that 78% of producers utilised ‘worm-safe’ pastures (defined as pastures rested for 4 months, or grazed by adult stock, or grazed by sheep, or used for hay and silage making, or used for crop making) within their gastrointestinal control programs.

Conclusion:
Meat and Livestock Australia currently recommends a drench at weaning (autumn) and in late winter, followed by a drench in spring if the winter drench was not a macrocyclic lactone and possibly a summer drench, for spring calving herds in the Central Tablelands¹. Only 24% of producers (10/41) are following these recommendations, and of these all but one producer is using a macrocyclic lactone.

References
GENOMIC COPY NUMBER VARIATIONS IN THE TASMANIAN DEVIL MAJOR HISTOCOMPATIBILITY COMPLEX

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Final year, full time.

Abstract
The Tasmanian devil (Sarcophilus harrasii) is currently under threat of extinction due to an unusual fatal contagious cancer called Devil Facial Tumour Disease (DFTD). DFTD is caused by a clonal tumour cell line that is transmitted between unrelated individuals as an allograft¹ without triggering immune rejection due to low levels of Major Histocompatibility Complex (MHC) diversity² in Tasmanian devils. In this study we characterized the genomic regions encompassing MHC Class I and Class II genes in the Tasmanian devil through bacterial artificial chromosome (BAC) contig construction and sequencing. Four genomic regions approximately 960kb in length were assembled and annotated. 34 genes and pseudogenes were identified, including five Class I and four Class II loci. Comparison between two haplotypes from two individuals revealed three genomic copy number variants with sizes ranging from 1.6 to 17kb within the classical Class I gene region. One deletion is particularly important as it turns an antigen-presenting Class I gene into a pseudogene in one of the haplotypes. The frequency of this deletion is highest in the northwestern devil population and lowest in southeastern areas. These copy number variants are likely to significantly affect the expression of adjacent MHC genes and therefore may have a critical role in the susceptibility/resistance of devils to DFTD.

References
EXPLORING ENDOGENOUS RETROVIRUSES IN THE CROCODILIAN GENOME

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Second year, full time

The saltwater crocodile (Crocodylus porosus) is one of two species of crocodile found in Australia and the only one that is commercially farmed. Runtism is one of the major causes of juvenile mortality, and is suspected to be related to inherent genetic factors, of which endogenous retroviruses might be a possibility. Endogenous retroviruses (ERVs) are inherited copies or remnants of exogenous retroviruses that have been integrated into germline cells and therefore passed on to subsequent generations. The broader scale of this project is to identify and characterise endogenous retroviruses in the saltwater crocodile and assess their significance for diseases in farmed populations. Here we will be discussing the screening and sequencing of full length ERVs from a saltwater crocodile genomic library.

DNA fragments from the retroviral pro-pol gene region were used as probes to screen a saltwater crocodile bacterial artificial chromosome (BAC) library. A BAC library from the gharial (Gavialis gangeticus), a sister subfamily to crocodiles, was also screened using the same probes as a control for comparison purposes. Over 800 clones containing potential ERV fragments were identified in the saltwater crocodile, with 48 producing very strong hybridisation signals. These 48 were purified and sequenced on the Roche GC FLX 454 sequencing platform. DNA sequence contigs will be assembled using the Newbler program by Roche. These contigs will be compared to known ERV sequences to identify full length ERVs and their insertion sites for further analysis.

Previous investigations into the ERV complement of crocodilians have revealed two major lineages of ERVs, one of which is unique to the family Crocodylidae (CERV1), and another which is common among a crocodilian species (CERV2). Comparisons between the hybridisation patterns in the crocodile and gharial BACs support these findings, with probes for CERV1 sequences hybridising only to the saltwater crocodile BAC library, while probes for CERV2 sequences hybridised to both libraries. In addition, we have identified ERV DNA sequences in existing crocodile genome data although the functionality and expression of these sequences have yet to be verified. The data available to date supports the notion that ERVs are present throughout the crocodile genome in many copies of both ancient and recent ERV insertions. Translated amino acid sequences from ERVs that show potential functionality will be used to assess whether those retroelements are expressed in saltwater crocodile tissues.

Acknowledgements

Many thanks to: David Ray and lab group, Travis Glenn and lab group, Daniel Peterson, Zenaida Magbanua

This project was funded by following grants: RIRDC PRJ-002461; and NSF MCB-084-1821 and DEB-102-0865

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Dog health in rural and remote Indigenous communities is on average poorer than suburban averages. Various strategies are being used to address this issue, including improving access to veterinary services, implementing education programs, and training local people in dog health work. This study compared programs across eight rural and remote communities nationally to explore the effect of veterinary programs, local health worker programs, and education programs working alone or in concert.

The study first undertook needs analysis and dog health surveys. Findings showed a lack of knowledge sharing, and a lack of engagement between service providers, organisers, and the community, as well as a lack of access to veterinary services, to be major factors in dog health issues. To address these findings, veterinary services were ensured, local animal health workers were trained or upskilled in areas such as parasite treatment and health promotion, and education programs developed and implemented.

Regression analysis found local health workers working alone produced variable results. On average, veterinary services produced lower prevalences of mange-like signs. However, the lowest prevalence of mange-like signs were found in communities which had veterinary services working together with local dog health workers trained in the theory and delivery of mange control. Programs with 50% or more of their face-to-face treatment team composed of local Indigenous people had significantly better results than teams with less.

Dog health programs that incorporated education programs based on identified local preferences achieved significantly better results in terms of improvements in mange prevalence and average condition score, partly through increased community understanding and engagement with the program.

Partnering with community needs and strengths in both veterinary service delivery and education programs significantly improved the success of dog health programs. Though more time consuming to organize and deliver, a collaborative approach delivers outcomes beyond the immediate scope dog health program and can develop the community’s capacity to care for themselves and their dogs into the future.

Acknowledgements

- Participants in training programs, community members, and their dogs
- ARC Linkage grant partners AMRRIC, RSPCA (NSW), Idexx Ltd.
COMPARATIVE PERFORMANCE OF FREE RANGE AND CONVENTIONAL BROILERS

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² Faculty of Science, University of New South Wales.

First year, full time.

Introduction

Free range broiler production is in its infancy in Australia but is growing rapidly. In 2006 free-range broiler production accounted for 4% of total broiler production and today it is around 15% ¹. Free-range broiler production is associated with poorer bird performance, high feed conversion and mortality compared with conventional broiler production. This ‘performance gap’ may be as a result of poorer digestive health, coccidiosis and dysbacteriosis challenge, nutritional inadequacy and variable pasture consumption. These performance challenges contribute to poor economic sustainability in the industry. It is the purpose of this paper to describe performance of free-range and conventionally-reared broilers at the same location, under commercial production constraints. Comparative benchmarking is an important prerequisite to further exploratory empirical or mechanistic research.

Materials and Methods

Two farms from the same geographical area (1.5 km diameter) were selected in order to minimize environmental differences. Placement of day old chicks and donor flocks were synchronized as much as possible. Both farm received the same diet from the same feed mill and the only difference was that the free-range diet did not contain in-feed antibiotics as per the requirements of FREPA. Dead birds were collected daily. Birds in both farms were weighed at 7, 14, 21, 28 and 35 days. Feed conversion was calculated at the end of each batch for each farm and corrected to 2.45 kg to be able to compare different killing age and final weights. Data were entered into an Excel spreadsheet and exported to JMP v.8 (SAS Software). Production system and age were used as leverage terms in a least square model to explore the main effects of both and interactions between the two on body weight and mortality. As FCR was only known for a whole batch (not by age) the main effect of production system only was explored. Significance was set at P<0.05 and where differences existed means were separated using Tukeys LSD.

Results and Discussion

There was a significant detrimental effect of free-range production on mortality and body weight gain. However, effect on body weight were most acute from d28-35 resulting in a significant interaction between age and production system (P<0.01). FCR corrected to 2.45kg body weight was significantly higher in free range compared with conventional broiler production. Impact of higher FCR in free range production in Australia is around $8,085,000 per year. While these costs are substantial, the higher mortality in free-range systems is an indication of strong disease challenge and/or metabolic disorders. Considerable demand exists for chicken meat that has been produced under free-range systems. This demand is partially emotive and linked to anthropomorphic interpretation of intensive animal practice. However, the performance gap is substantial and not sustainable in the long-term. The reasons for this performance gap are obscure and further research is required to delineate the effects of the absence of in-feed antibiotics and the effects of range access. A greater appreciation for the challenges that free-range broilers face, whether immunological, nutritional or behavioural, will allow more appropriate and strategic intervention by producers on one or all of these axes.

Acknowledgements

Noelene Lewis, Graeme Stewart and Mark Attard, Red Lea Chickens Free Range and Conventional Broiler Farms, Mandalong, NSW, Australia.

References

THE EFFECT OF TOPICAL ANAESTHESIA AND XYLAZINE ON THE PAIN SENSITIVITY RESPONSES OF LAMBS UNDERGOING CASTRATION AND TAIL DOCKING

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Currently 3rd year (Full Time)

Abstract

Castration and tail-docking are routine husbandry procedures performed in Australia’s wool industry to eliminate unwanted mating and prevent dag formation, respectively. Despite the method used to perform these procedures, changes in lamb physiology and behaviour indicate these procedures are highly likely to be painful¹. The provision of pain relief is an important tool to help improve the welfare of animals subjected to these procedures however providing effective, practical and affordable pain relief remains a challenge given the financial constraints imposed on commercial animal production. The aim of this study was to examine the efficacy of two relatively practical pain relief compounds in alleviating the pain induced by castration and tail-docking in lambs. Forty-eight (n = 48) 6-8 wk old lambs were randomly assigned to one of six treatment groups: sham marking (marking = surgical castration + tail-docking with a hot-iron), sham marking with a pre-operative injection of xylazine (intra-muscular at 0.5mg/kg), marking alone, marking with a pre-operative injection of xylazine, marking with a post-operative application of topical anaesthesia (Tri-Solfen®, Bayer Animal Health), and marking with a pre-operative injection of xylazine and post-operative application of topical anaesthesia. The efficacy of the pain relief compounds was assessed by performing quantitative sensory testing which aims to assess wound and skin sensitivity by gauging the animal’s response to mechanical stimulation. Von Frey monofilaments (10 and 75g) were used to stimulate sites within and around the wounds, and head and rump involuntary reflexes and motor responses were categorised based on a numerical scale where 0=no response, 1=minor response, 2=moderate response and 3=severe response. This was performed prior to marking and 30 min, 6 and 24 h post marking. Data is currently under analysis and will be presented at the conference.

Acknowledgements

Peter Thomson for statistical advice.

References

T-CELL RECEPTOR GENE REARRANGEMENTS FOR CHARACTERISATION OF LYMPHOCYTE CLONALITY IN CATS WITH LOW GRADE ALIMENTARY LYMPHOMA.

Evans NA\(^1\), Beatty JA\(^1\), Uvjari B\(^2\), Briscoe KA\(^1\), Belov K\(^2\), Barrs VR\(^1\)

\(^1\)The Valentine Charlton Cat Centre, Faculty of Veterinary Science, The University of Sydney
\(^2\)Faculty of Veterinary Science, Sydney
Second year, full time.

Introduction
Lymphoma is the most common form of cancer in cats, with alimentary lymphoma (AL) the most common anatomical form. Recently, a unique subtype of AL was identified – low grade alimentary lymphoma (LGAL). Diagnosis of LGAL may be delayed or confused because of clinical and histological similarities to non-neoplastic diseases, principally inflammatory bowel disease (IBD). Thus alternative methods are required to differentiate neoplastic from inflammatory lymphocyte populations in the feline intestine. One feature that lymphoid malignancies share is the expansion of a clone of lymphocytes. There may be a single neoplastic clone (monoclonal) or several clones (oligoclonal). Lymphocyte clonality can be assessed by amplification of the T-cell receptor gamma gene (\textit{TRCG}) via use of the polymerase chain reaction (PCR) to look for specific genetic rearrangements suggestive of a monoclonal population of lymphocytes. Because \textit{TCRG} has limited diversity a limited primer set can be used. The aim of this project is to characterize the clonality of lymphocytes in formalin fixed paraffin embedded intestinal biopsies (IB) from cats with diagnosed IBD or LGAL, and to compare results of clonality testing to histopathological diagnosis and immunophenotype.

Materials and Methods
Archival IB from 53 cats previously diagnosed with LGAL (n=29) and IBD (n=24) were retrieved. DNA extraction was performed using a commercially available kit after paraffin extraction (DNeasy tissue extraction kit, QIAGEN). PCR was performed using five different primer sets, consisting of one set of consensus primers and four sets of standard primers previously published by Moore et al 2005 and Weiss et al 2011. 100ng of genomic DNA was amplified in a 50ul reaction, and a 2 step modified touchdown protocol was used. All samples were run in triplicate, with sterile water used as a negative control. Products were visualized using polyacrylamide gel electrophoresis (PAGE), and triplicate samples were run side by side for comparison. The presence of one to two sharp bands (monoallelic or biallelic) of similar size and shape present in triplicate samples confirmed the presence of a clonal sample. If three to five sharp bands were present and reproducible in triplicate samples then the result was considered to be oligoclonal, while the presence of a broad band, ladders of bands or smearing was considered to be polyclonal. A sample was considered to be pseudoclonal if bands of disparate size were present in triplicate analysis. Heteroduplex analysis was performed using denatured samples that were allowed to anneal prior to PAGE. This was done to confirm that bands consisted of molecules of identical size and sequence.

Results
Pending
THE EFFECT OF HUMIDITY, AIR TEMPERATURE AND WIND ON THE LOCAL SPREAD OF EQUINE INFLUENZA

Simon Firestone¹, Naomi Cogger², Barbara Moloney³, Jenny-Ann Toribio¹, Michael Ward¹ and Navneet Dhand¹

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² EpiCentre, Institute of Veterinary, Animal, and Biomedical Sciences, Massey University, Palmerston North, New Zealand
³ Department of Primary Industries, NSW Trade and Investment, Regional Infrastructure and Services, Orange, NSW, Australia

Third Year PhD research (currently full time)

Abstract

The influence of relative humidity and ambient temperature on the transmission of influenza A viruses have been established under controlled laboratory conditions. The interplay of meteorological factors during an actual influenza epidemic is less clear, and research into the contribution of wind to epidemic spread is scarce. We quantified the association between hazard of infection and environmental risk factors (such as air temperature, relative humidity and wind velocity) by applying geostatistics and survival analysis on data from the largest cluster of the 2007 epidemic of equine influenza (A/H3N8) in Australia. Meteorological conditions at each premises location were estimated by smoothing concurrent daily meteorological data, and analysed as time-varying time-lagged covariates using generalised Cox regression. Wind velocity and minimum daily air temperature time-lagged by 2 – 3 days were strongly associated with an increased risk of infection, corresponding closely with the incubation period of equine influenza. An interaction was detected between relative humidity and air temperature (p<0.01), and strong winds (> 30 km hour⁻¹) from within a 45° arc centred on the direction of the nearest potential source premises were also strongly associated with increased risk of influenza infection, 3 days later (p=0.02). Through combining influenza outbreak surveillance and concurrent meteorological data, we provide empirical evidence for the underlying environmental mechanisms that influenced the spread of this equine influenza epidemic. Our analyses support, and extend, the findings of studies into influenza A transmission conducted under laboratory conditions. The relationships described are of direct importance for managing disease risk during influenza epidemics in horses, and more generally, may inform the modelling of epidemics of other infectious diseases of both humans and animals.

Acknowledgements

This research is jointly funded by Rural Industries Research and Development Corporation (RIRDC) and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Diseases (ABCRC).

References

OLIGODENDROCYTE LOSS IN THE LYSOSOMAL STORAGE DISEASE CANINE FUCOSIDOSIS

Jessica Fletcher, Gauthami S. Kondagari, Peter Williamson and Rosanne Taylor
Faculty of Veterinary Science, The University of Sydney.
Third year, full time.

Introduction
In the lysosomal storage disorder fucosidosis, myelin loss begins before the onset of clinical signs and is part of a pathogenic cascade involving neuronal vacuolation, death and activation of neuroinflammatory pathways\textsuperscript{1, 2}. Recent gene expression studies in canine fucosidosis have shown that oligodendrocyte (OL) and myelin specific genes are significantly downregulated in the cerebral cortex of 4 month old fucosidosis pups\textsuperscript{3}. This suggests that lysosomal storage impacts on OL development and survival. It is not known if this is due to lysosomal vacuolation within OLs or a secondary mechanism interfering with the axon-glial interaction required for OLs to reach maturity.

Materials and Methods
Brain tissue from fucosidosis-affected and age-matched controls dogs were examined using immunohistochemistry and electron microscopy (EM). Groups were as follows: Preclinical (n = 6), Early (n = 6), and Late (n = 6) disease, Control pups (n = 3) and adults (n = 8). EM was used to identify lysosomal vacuolation in OLs. Immunohistochemical stains included Apoptag TUNEL for apoptosis, CNPase for mature OLs and neurofilament (NfL) for axons. Positive cell counts and quantification was performed using image analysis.

Results and Discussion
All OLs identified by EM in preclinical fucosidosis dogs had numerous vacuoles with stippled contents within their cytoplasm. Preliminary results indicate that CNPase\textsuperscript{*} OLs are significantly (P = 0.01) reduced in the cerebral cortex in affected dogs in preclinical, early and late disease, explaining previous findings of reduced expression OL specific genes in preclinical disease\textsuperscript{3}. There is a trend towards increased apoptosis of cerebrocortical white matter glia in preclinical affected dogs. Suggesting that this is the mechanism by which OLs are lost at this stage in disease progression. Myelin loss becomes more severe in late disease\textsuperscript{2} and significant (P <0.001) reductions in the percentage of NfL\textsuperscript{*} staining in only late fucosidosis dogs suggest that it is driven by axonal degeneration. These findings suggest that early lysosomal storage impacts OL populations in preclinical disease before the development of secondary inflammatory neuropathology and neuronal loss exacerbate early myelin loss in late disease.

Acknowledgements
The authors are grateful to Elaine Chew and Karen Barnes at Veterinary Pathology Diagnostic Service, Faculty of Veterinary Science, The University of Sydney and Delfine Cheng and Shaun Bulcock at the Australian Centre for Microscopy and Microanalysis for training, assistance and technical advice. The authors also acknowledge the facilities as well as scientific and technical assistance from staff in the AMMRF (Australian Microscopy & Microanalysis Research Facility) at the Australian Centre for Microscopy & Microanalysis, The University of Sydney.

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\textsuperscript{3} Fletcher J.L., Kondagari G.S., Wright A.L., Thomson P.C., Williamson P. & Taylor R.M. In press ‘Myelin genes are downregulated in canine fucosidosis,’ Biochimica et Biophysica Acta – Molecular Basis of Disease, doi:10.1016/j.bbadis.2011.06.001
ORIGIN OF APICOMPLEXAN FLAGELLA: MORE QUESTIONS THAN ANSWERS, YET HOPE

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First year PhD (full time)

The Apicomplexa, a group of intracellular parasitic protozoans, evolved from an ancestral organism possessing flagella. However, this locomotory appendage has been lost except for the male microgametes of certain genera, where it plays a role in their sexual reproduction. Eukaryotic cilia and flagella are composed of a membrane-ensheathed axoneme made of a specialised arrangement of microtubules, and are assembled by the evolutionarily conserved process of ‘intraflagellar transport’ (IFT). IFT protein homologues have been identified in a diverse range of eukaryotes, though apicomplexans present an intriguing case. For example, Eimeria and Toxoplasma possess only a subset of IFT proteins, while Plasmodium lacks IFT proteins altogether. In fact, Plasmodium microgamete flagella production is unique in that it occurs completely intracytoplasmically via an extremely rapid, uncharacterised IFT-independent mechanism¹. This project aims to demonstrate how Chromera velia – a free-living ancestor of Apicomplexa that was recently discovered in Australian corals – may be used to unmask the flagellar assembly process in Apicomplexa, and to increase understanding of its role during the development of a parasitic lifestyle. Gene expression dynamics of C. velia flagellar structural and functional components will be analysed by qRT-PCR at various stages of its lifecycle, and immunolocalisation will be employed to investigate flagellar assembly within C. velia cells.

References
MEGALOCYTIVIRUS TRANSMISSION BETWEEN FISH POPULATIONS USING THE EURYHALINE SPECIES AUSTRALIAN BASS, *MACQUARIA NOVEMACULEATA*, AS AN EXPERIMENTAL VECTOR

Go J and Whittington R

Second Year PhD research
Faculty of Veterinary Science, University of Sydney, NSW 2006, Australia

Abstract

Initial findings from a mass mortality event of Murray cod (*Maccullochella peelii peelii*) fingerlings during February 2003 at a Victorian aquaculture facility suggested infection with a group of viruses previously not known to be established in Australian aquaculture. Subsequent research demonstrated involvement of a megalocytivirus with strong similarities to infectious spleen and kidney necrosis virus (ISKNV) and dwarf gourami iridovirus (DGIV). Murray cod have also been shown to be highly susceptible to a megalocytivirus isolated from imported dwarf gouramis (*Colisa lalia*) in experimental transmission trials. However, it is unclear if other Australian native fish species are similarly susceptible. Australian bass, *Macquaria novemaculeata*, is a native Australian euryhaline species belonging to the family Percichthyidae. Two members of this family have previously been shown to be highly sensitive to megalocytiviruses of the ISKNV group. Australian bass was therefore chosen as a potential candidate model vector for transmission of megalocytivirus between fish populations. A series of experimental transmission trials was undertaken to test the susceptibility of Australian bass to megalocytivirus infection and ability to transmit infection to Murray cod. Australian bass fingerlings that were intraperitoneally (IP) injected with a megalocytivirus isolated from dwarf gourami, DGIV-10, or cohabited with Murray cod inoculated with DGIV-10 exhibited high levels of morbidity with affected fish generally succumbing within four weeks post inoculation. In both cases, clinical signs consistent with those observed in infected Murray cod were observed shortly before fish became moribund. When IP injected Australian bass, and Australian bass cohabitated with infected Murray cod, were exposed to naïve Murray cod juveniles, this resulted in very high morbidity rates in these naïve juveniles. Again, clinical signs of megalocytivirus infection were observed. Quantitative polymerase chain reaction results, representative histology and associated in situ hybridization analysis provided further support that transmission of megalocytivirus infection had occurred. This suggests that Australian bass may be a suitable model for studying transmission of megalocytivirus infection.

References

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THE EFFECTS OF GRAIN, FRUCTOSE AND HISTIDINE ON INFLAMMATORY AND OXIDATIVE STRESS RESPONSES IN DAIRY HEIFERS IN AN INDUCED SUBACUTE ACIDOSIS PROTOCOL

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PhD, Second year, Full time

Introduction

Experimentally induced subacute acidosis increases oxidative stress in dairy cattle1. Our subacute acidosis study showed feeding grain, or the substitution of fructose for grain, decreased ruminal pH and increased volatile fatty acid concentrations. In fructose substituted animals lactic acid concentrations were also increased. We investigated the effects of feeding grain, fructose and histidine on inflammatory and oxidative stress responses in dairy heifers.

Materials and Methods

Thirty Holstein heifers were randomly allocated into 5 challenge treatment groups; 1) Control (no grain), 2) Grain (1.2% liveweight (LW)), 3) Grain (0.8% LW) + fructose (0.4% LW), 4) Grain (1.2% LW) + histidine (6 g/hd) and 5) Grain (0.8% LW) + fructose (0.4% LW) + histidine (6 g/hd) in an incomplete factorial design. Blood samples were collected at 5 and 215 minutes after treatment consumption and analysed for plasma reactive oxygen metabolites (ROMs), biological antioxidant potential (BAP), oxidative stress index (ROMs/BAP), advanced oxidation protein products, ceruloplasmin and glutathione peroxidase activity.

Results and Discussion

Dietary treatment had no significant effect on inflammatory or oxidative stress biomarkers. Concentrations of ROMs and the oxidative stress index increased over the sampling period, from 108.7 to 123.5 Carr.U and from 4.1 to 4.8 respectively (P=0.002 and 0.009). Ceruloplasmin concentrations markedly declined over time from 0.19 to 0.09 g/L (P<0.001). Overall, despite elevated lactic acid concentrations and marked changes in ruminal metabolism during this subacute acidosis challenge no clinical signs of acidosis or marked inflammatory or oxidative stress responses in grain, fructose or histidine fed heifers were observed. Suggesting ruminal conditions were not sufficient to induce responses.

References

**Lawsonia intracellularis** VACCINE DOSE AND ROUTE ON PROLIFERATIVE ENTEROPATHY PROTECTION FOLLOWING CHALLENGE IN PIGS

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²Department of Primary Industry NSW, Elizabeth Macarthur Agricultural Institute, Menangle.
Second year, full time.

**Introduction**

Proliferative enteropathy (PE) is an important economic disease of pigs, and is caused by the intracellular Gram-negative bacterium *Lawsonia intracellularis*. This agent colonizes intestines and can develop clinical signs of diarrhoea and weight loss in pigs of all ages. Vaccination with a live oral vaccine (Enterisol®Ileitis)¹ or a *L. intracellularis* bacterin vaccine administered intramuscularly² can prevent or control disease. However, characterisation of mucosal immune responses to *L. intracellularis* is very limited. This project ultimately aims to identify an immune marker which correlates with protection against PE following vaccination. So, we aimed to model the immune response associated with different dose and route vaccination in pigs challenged with a virulent form of *L. intracellularis*.

**Materials and Methods**

Forty six weaner pigs (6.0±0.5 kg) were allocated into each of 4 test groups (n=10), Positive control (PC), one-time oral (1xOR), one-time intramuscular (1xIM), ten-time oral (10xOR), and six pigs for the negative control (NC) group. Pigs at 5 weeks of age were given a commercial *L. intracellularis* vaccine (day 0) with single or ten-times dose by oral or intramuscular (IM) route. Pigs were challenged with an oral inoculum of 10⁹ *L. intracellularis* from infected intestinal mucosa at day 21 post-vaccination (p.i.). Pig weights were recorded on day 0, 21 and 42 to determine the average weight gain. Blood was collected weekly to test for *L. intracellularis* specific IgG and IgA. The number of *L. intracellularis* shed in faeces was estimated using quantitative PCR³. Animals were necropsied 21 days after challenge, and intestinal tissues were stained by H&E to determine lesion severity and the amount of *L. intracellularis* present was detected by immunohistochemistry (IHC).

**Results and Discussion**

We were able to reproduce PE infection in pigs after challenge with *L. intracellularis*, as demonstrated by seroconversion, *L. intracellularis* shedding in faeces and intestinal lesions. All pigs appeared clinically normal with no diarrhoea throughout the trial. However, we observed significant reductions in intestinal lesions in vaccinated pigs compared with non-vaccinated pigs. In addition, we demonstrated that pigs vaccinated shed lower number of *L. intracellularis* (10⁵) in faeces when compared with non-vaccinated pigs (10⁶) after two weeks challenge. There was no difference (p>0.05) in antibody concentrations of timing of seroconversion between treatment groups. Overall, this study demonstrated that animals treated with a 10x oral vaccine dose were better protected against *L. intracellularis* challenge than pigs vaccinated with a single dose orally. There was no significant difference in protection between pigs vaccinated orally or IM with a single dose of Enterisol, supporting reported success with a bacterin².

**References**

IMPROVING ACCESS TO GENOMIC RESOURCES FOR NON-COMMERCIAL SPECIES: APPLICATIONS FOR CONSERVATION.

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First year, full-time.

Abstract

Recent progress in mammalian sequencing projects and focused SNP discovery has facilitated the creation of large-scale genotyping arrays for many commercially significant species. The expense associated with the production of these high-density arrays has thus far prevented the wider application of this technology in conservation-based projects. The broad aim of this project is to investigate the potential utility of existing genotyping arrays to provide useful information in under-resourced species. For example, by leveraging the existing domestic horse SNP array, what practical genetic data may be generated to assist in the conservation of threatened zebra species? Predictions state an extremely small number of SNPs should be present as shared polymorphisms between diverged species. Despite this, several studies have observed surprisingly high numbers of SNPs from the array population segregating in wild relatives. This suggests shared polymorphisms in extant species may be ancestral in origin, a condition referred to as phylogenetic inheritance. Establishing collections of truly polymorphic markers will be valuable for measurements of population genetic diversity, and for establishing relationships in captive breeding programs.

This project currently has access to cross-species genotyping data from equine, ovine, and bovine arrays, generated using the Illumina 50K beadchip platform. SNP calling rates, polymorphism, and dispersion in test species will be determined through computational analysis and mapping onto the array species genome. Strategic sampling of DNA from non-array species will be undertaken to identify truly polymorphic SNPs and expand on theoretical predictions. The potential applications for conservation and population genetic analysis will be explored.

Preliminary assessment of data from the equine array has revealed promising evidence of informative SNP markers in non-horse relatives. Genotyping at 54602 loci returned call rates averaging 95% in Equus species. Further, 15% of loci displayed both alleles in non-horse species, indicative of ancestral polymorphisms. These results support the potential use of existing genotyping arrays as a simple and cost effective way to approach conservation in non-model species.

References


IMMUNOGENICITY EVALUATION OF IN SILICO IDENTIFIED MAP RECOMBINANT PROTEINS THAT WERE UPREGULATED UNDER STRESS CONDITIONS

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Third year, full time

Introduction

\textit{Mycobacterium avium} subsp \textit{paratuberculosis} (MAP) is the causative agent of Johne’s disease (JD) in ruminants. It is known to enter a dormant phase outside the host typically on soil \textsuperscript{1}. Survival inside the host macrophage is a hallmark of MAP infection and dormancy may play a role in this survival. \textit{In vitro} experiments have reported regulation of certain MAP proteins when exposed to stressors similar to dormancy \textsuperscript{2}. It is believed that \textit{in vivo} regulation of dormancy genes and associated proteins by MAP may play an important role in evading the host defence mechanisms and the host may also mount an immune response against these dormancy related proteins. Evaluation of such proteins may provide insight to host-pathogen interaction during the course of MAP infection.

Materials and Methods

A group of dormancy genes upregulated under stress conditions were examined using \textit{in silico} analysis to identify B and T-cell epitopes. Five potential candidate genes based on epitope prediction results were selected and cloned: three hypothetical proteins and two proteins involved in fatty acid metabolism. Recombinant proteins were produced, purified and evaluated for their immunogenicity using a panel of sera from sheep with a spectrum of JD and sheep free of MAP infection, by detection of host specific antibodies in ELISA.

Results and Discussion

Individually, the five proteins were found to have the ability to discriminate between sera from sheep unexposed and exposed to MAP infections. \textit{In silico} analysis of genes is a rapid approach for functional characterisation and discovery of novel antigens for MAP diagnosis.

References

MYXOSPOREAN PARASITES IN AUSTRALIAN FROGS: IDENTIFICATION OF NEW SPECIES, RE-WRITING HISTORY, AND ELUCIDATION OF A THREAT TO FROG CONSERVATION

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Final year PhD, full time

A single myxosporean (Myxozoa) parasite was thought to infect Australian frog gall bladders with little host impact, this was identified in the 1980’s as *Myxidium immersum*, a parasite infecting Cane toads in South America. It was assumed that the Cane toad had introduced this parasite when it was released in northern Queensland in 1935. The Cane toad was translocated throughout the Caribbean before being bred up in Oahu, Hawaii and then shipped to Australia and throughout the Pacific. We used museum specimens and sampled toads in Hawaii (the intermediate location) to test the hypothesis that the Cane toad brought this parasite to Australia. Our results indicate that the Cane toad is innocent, rather than being the vector for this parasite into Australia it is possible that it has been hijacked by myxosporea and plays more of a role in the amplification of this parasite in the environment rather than the expansion of the parasite’s range. A further twist to the story is that through genetic and morphological identification and description we have determined this “*Myxidium sp*” infection as two distinct species sharing a common cryptic spore morphology. Myxosporean parasites develop within a vertebrate host to produce transmissible myxospores, the species in Australian frogs were thought to be a single species based on their common myxospore morphology. In fact our research has shown unprecedented diversity and revealed aspects of amphibian-myxosporea biology never seen before. The identification of these parasites led to discussion about their impact on Australian frog health and the potential for them to become a conservation threat. We have described the intra-tissue development in brains and livers of tadpoles and frogs in multiple amphibian species. The disease we have identified has significant potential to be a conservation threat for some populations of endangered frogs including the Green and golden bell frog, Booroolong frog and the newly re-discovered Yellow Spotted bell frog.
SADDLE PAD USAGE AND BEHAVIOUR AMONGST EQUESTRIAN RIDERS.

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PhD. Third Year. Full time.

It appears to be common practice to use a layer of cloth or similar between the saddle and the horse’s back [1]. Saddle pads of differing types and materials influence the amount and distribution of pressures recorded under saddles [2, 3]. There is little evidence that saddle pads can correct poor saddle fit [4]. Increasingly, saddle fit is being studied with pressure detecting devices so knowledge of common usage of layers between horse and saddle will align research with real world practices.

We designed an online survey (www.surveymonkey.com) to characterise saddle pad usage in the general equestrian community. We also asked participants to comment on the behaviour of the saddle pad/blanket during and after the ride.

Of 1001 responses, 42% of respondents nominated dressage as their main riding activity followed by 25% trail riding. Other activities well represented included eventing, showing, show-jumping, endurance, and working cattle. The vast majority of respondents (98.6%) used some form of layer between the saddle and their horse’s back and, of these, 84.5% pulled the layer up into the gullet of the saddle. 87.3% nominated the perceived horse’s comfort as a reason for using a layer. Just under half (43.5%) claimed that the layer descended onto or near the spinous processes during riding activities. Of the 54.1% of respondents who used more than one layer under their saddles, 64.6% answered that the resulting thickness of the layers was greater than 1cm.

These results indicate that usage of at least one layer between saddle and the horse’s back is commonplace in this population of riders. They have significant implications for any research on the ridden horse’s back as layers of material under the saddle may disperse or concentrate the pressures. Use of saddle blankets/pads is to be encouraged during pressure measurement so that research reflects reality. Pressure over spinous processes due to slippage behaviour in layers is an issue that demands both researchers’ and manufacturers’ attention, as it reduces the effectiveness of a saddle in distributing weight away from the dorsal midline. Increasing thickness of layers under saddles can increase horse discomfort, so the prevalence of retro-fitted padding suggests a need for better saddle fitting.

References:
DICEPHERING LEG CUES OF HORSE RIDERS.

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PhD. Third year. Full time.

Medially directed pressure cues from the riders’ legs (“leg aids”) are an almost universal signal for acceleration across equestrian disciplines. Riders use their legs to generate pressure against the horse’s thorax as part of the communication system between rider and horse. The rider then releases the pressure as soon as the desired response is obtained so that the horse is rewarded for the correct response.

We have developed a system for measuring critical variables involved in these signals: pressure, contact area, and duration of cue application.

Twelve riders of varying experience seated on a saddled artificial horse torso were asked to give a cue for “trot” they would usually give a well-trained horse every 20 seconds. This process was repeated 10 times over 200 seconds for each rider.

REML analysis of calf-contact-area data during cueing showed that inter-rider variance accounted for 89.7% of total variance but that intra-rider variance was small (1.7%) . Variance between applying and not applying the cue for each rider was 8.6% of total variance (p<0.001). Inter-rider variability in duration of change in calf pressure accounted for 72.1% of total variance; the remainder coming from intra-rider variability.

69.1% of total variance in calf pressure increases during cueing came from inter-rider variance. Variance between applying aid and not applying aid was 19.2% while intra-rider accounted for 11.7% of total variance (p<0.001). Duration of change in calf pressure indicated nearly equal intra-rider (48.4%) and inter-rider (51.6%) variance.

In this model, these data suggest that, for an individual rider, changes in calf-contact-area cues for an upward transition may be more consistent than calf pressure changes but that, between riders, calf pressure changes are more consistent. Durations of intra- and inter-rider change for both calf pressure and calf-contact-area were inconsistent.

This investigation has:
• Isolated changes in rider leg cue variables without the signal noise created by horse movement and asymmetry
• Identified the probable parameters that provide relevant stimuli in rider’s leg cues for the horse.
• Established baseline measurements for the riders’ leg cues.
• Identified sources of variability and therefore confusion in the upward transition cueing process

Further research will characterise leg cues in optimal riding technique.
DEVELOPMENT OF A LACTATIONAL OESTRUS INDUCTION PROTOCOL THAT CAN BE IMPLEMENTED IN CONFINEMENT FREE SOW HOUSING SYSTEMS.

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First year, full time.

Introduction

Based on the previous studies of Downing et al, it is known that oestrous can be induced in sows during lactation under a normal commercial environment (2009)¹. From this, it is possible to synchronise sows oestrous to improve productivity and eliminate excessively long weaning to oestrus interval. This project aligns with the objective of the new Pork CRC of “viable mating of sows in confinement free farrowing/lactational housing systems” by July 2014.

Materials and Method

A recent trial at Rivalea Corowa involved three treatment groups; controls (A), treatment with PMSG and hCG shortly after farrowing followed by boar exposure (B), and exposure to boar contact only (C). The latter two treatment groups were also tested twice daily for standing oestrus and blood samples (Progesterone). All sows were culled 4 days after weaning and the ovaries collected for gross anatomical analysis. The subsequent investigations will include rectal ultrasound to monitor follicle development and spontaneous oestrus in control sows, sows exposed to boar exposure only and sows that have had 3 days of either 8 or 16 hour piglet separation at day 21 of lactation. In addition ultrasound will be used to track the growth of the ovaries in sows during lactation under different suckling loads to establish a protocol for the best time to induce ovulation and timed insemination.

Results and Discussion

The trial completed earlier in 2011 has indicated that 16, 41 and 23% of treatment A, B and C sows ovulated during lactation, respectively. The 16% of the controls are believed to equal the number of sows recorded by many producers to be unmated 7 days after weaning and account for a huge proportion of sow wastage within a herd. There is a need to overcome this issue and develop strategies that can be adopted by producers that will increase their productivity both efficiently and economically.

Acknowledgments

Pork CRC, for funding the project, staff of Rivalea Corowa for assistance with labour and blood collection and staff of Diamond Valley Pork for help with collection of ovaries.

References

INVESTIGATION INTO THE SURVIVAL OF CRYOPRESERVED BIOPSIED SHEEP EMBRYOS FOR MARKER ASSISTED SELECTION AND EMBRYO TRANSFER.

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First year, full time.

Introduction
Marker assisted selection (MAS) is a process in which different markers are used to identify the genetic trait of desired selection and the selection of individuals to become the parents for the next generation. This is of particular advantage for traits which show late expression in development, age, sex or species specific and difficult to measure at an early age. Therefore, biopsied embryos serve as an early source for the genetic evaluation (MAS) and increase the rate of genetic gain by significantly reducing the generation interval. Factors affecting the survival of biopsied sheep embryos for marker assisted selection are yet to be investigated. The present study aims to establish the optimum manipulation and handling conditions for growth and survival of cryopreserved biopsied sheep embryos. The outcomes of the present study will inform the development of animal breeding programs that incorporate marker assisted selection of embryos.

Materials and Methods
Embryos at the blastocyst stage will be produced both in vitro (using different growth promoters) and in vivo, biopsied and cryopreserved at different times (0, 2, 3, 5 and 10 h) following biopsy. The biopsied, cryopreserved embryos will then be thawed and cultured to access embryo survival. Using the optimal conditions, biopsied and cryopreserved embryos will be transferred to recipient animals (synchronised to the relative developmental stage) and pregnancy outcomes at 50 days of gestation and at parturition will be recorded. The survival of in vitro and in vivo produced embryos will also be compared under different conditions.

Acknowledgements
Staff of Picton Abattoir, Koorana Road, Tahmoor NSW 2573

References

DEVELOPMENT OF DIRECT AND INDIRECT ELISA FOR NATIVE NERVOUS NECROSIS DIAGNOSIS

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First year PhD student, full time

Introduction

Viral Nervous Necrosis (VNN) is a disease causing significant losses in marine aquaculture worldwide including Australia\(^1\). Affected fish usually display abnormal swimming behaviour and finally death with mortalities of up to 100%\(^2\). Although, transmission of the virus is thought to be both horizontal and vertical, doubt has been cast on the evidence supporting vertical transmission of the disease\(^3\). Understanding transmission is crucial for prevention policies and the development of a serological test for broodstock screening is considered to be an important step because it may reveal a source for vertical transmission. Indirect ELISA will enable testing of adult fish for virus exposure-immune status assessment without causing any harm to the fish itself. Also, direct ELISA could provide for accessible and cheap detection of the viral antigen in fish tissues and cell cultures for diagnostic laboratories.

Materials and methods

Fish sera: Positive controls were obtained from serum samples of Barramundi \textit{Lates calcarifer} and Australian Bass \textit{Maquaria novemeaculata} vaccinated with NNV recombinant coat protein (rcp).

Antigen: NNV reference isolate was grown in SSN-1 cells and clarified tissue culture supernatant (tcsn) was used as antigen for antibody capture in indirect ELISA and for positive controls in direct ELISA.

ELISA: a sandwich ELISA using sheep anti-NNV affinity purified antibodies as the capture system is being optimized.

Results and Discussion

Virus presence in tcsn was detected by direct ELISA. The best assay format was the sandwich ELISA using sheep anti-native NNV as both primary and secondary antibody. Using indirect ELISA, barramundi anti-NNV rcp antibodies could be detected, but Australian bass serum bound non-specifically to the capture system and further experiments are needed in order to understand this observation and develop a solution.

Acknowledgements

Alison Tweedie, The University of Sydney Aquatic Animal Health Unit provided assistance with cell culture.


MHC CLASS I DIVERSITY IN SALTWATER CROCODILES AND ITS ASSOCIATION WITH LYMPHOID PROLIFERATION, VASCULITIS AND ENCEPHALITIS SYNDROME
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Third year, part time since semester 1, 2011.

Introduction
Lymphoid proliferation, Vasculitis and Encephalitis (LVE) syndrome has recently been the cause of high mortalities of farmed saltwater crocodiles (Crocodylus porosus) and is presumed to have a viral aetiology. Reports have suggested that some instances of susceptibility to infectious diseases in individuals could be associated with low or lack of genetic diversity of the major histocompatibility complex (MHC) genes [1]. The MHC class I genes are highly polymorphic and involved in the adaptive immune response to viral infection. In addition, previous studies have suggested that different variants of these MHC class I genes could recognise similar peptide antigens as they show similarities between putative structure and function in their peptide binding regions (PBR) [2]. This has been used to allow them to be grouped into super-type variants with relevance to assess disease and MHC association. Here, we assess whether the distribution of specific gene variants or super-types of MHC class I PBR (exons 2 and 3) segregate according to the presence of LVE syndrome.

Materials and Methods
• 77 yearling saltwater crocodiles were selected at random from ten similar pens in one area of the farm, and were classified as diseased (n = 30) or healthy (n= 47) using results from physical examination. Of the 7 diseased animals subjected to histopathology, all had signs of LVE, and of these 2 cultured herpesvirus.
• MHC class I exons 2 and 3 in each crocodile were amplified and sequenced using 454 Next Generation Sequencing. In order to minimise artifacts, two independent PCR reactions from each individual were performed. True gene variants were called when they fell into mean and maximum frequency criteria of the least abundant consensus sequences between the two replicates within an individual.
• Gene variants were translated into amino acid (aa) sequences and only those showing functional open reading fragments were included in the analyses. Clustering of variants at the aa sites under positive selection into super-types was assessed using Maximum Likelihood and Hierarchical Clustering analyses [3]. The distribution of MHC polymorphism in each exon was compared between the two animal groups using the Fisher's exact test and an odds ratio.

Preliminary results
• A total of 96 MHC class I exon 2 variants and 12 exon 3 true variants were identified. These variants clustered into 13 and 4 super-types, respectively.
• Interestingly, an exon 3 variant and the super-type to which it belongs appear to be significantly associated with the diseased animals. It may be possible to suggest susceptibility of an individual carrying that particular variant/super-type to the disease.

References
DISPOSITION KINETICS OF MELOXICAM FOLLOWING INTRAVENOUS, SUBCUTANEOUS AND ORAL ADMINISTRATION IN THE KOALA

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One of the major mortality in koalas (Phascolarctos cinereus) is injuries inflicted by cats and dogs attack or motor vehicle accidents. Meloxicam (MEL), a non-steroidal anti-inflammatory drug with preferential cyclooxygenase-2 inhibitory activity, has been commonly used in these traumatized koalas. Up to date, no pharmacokinetic information of MEL in koalas has been reported; current dosage regimen in koalas is extrapolated from the dose established for other species (0.1-0.2 mg/kg, oral, and s.i.d). In adult people with this dosage regimen, oral bioavailability of MEL is ≈ 89 % and elimination half life (t1/2) is ≈ 20 hr. The aim of this study was to evaluate the pharmacokinetic of MEL in koalas. From clinically healthy koalas, after dosing with MEL (metacam) via intravenously (n=5, 0.4 mg/kg), subcutaneously (n=3, 0.1 mg/kg) and orally (n=3, 0.1 mg/kg), blood samples were collected at different times. Plasmas from collected blood samples were cleaned with solid phase extraction and the MEL plasma concentrations were quantified with validated high performance liquid chromatography equipped with diode array detector. Pharmacokinetic indices were estimated based on the non compartmental analysis. Results of the current study indicated that koalas appeared to have minimal oral bioavailability of this drug (subcutaneous bioavailability ≈ 70 %) and following the intravenous bolus injection, approximately 50 % of MEL in the plasma decreased after ≈ 65.26 min (t1/2) due to extremely fast plasma clearance rate of this drug. From the PK perspective, current MEL dosage regimen (eg. route of administration and dosing interval) appeared to be inadequate to produce significant therapeutic effect in koalas.

Acknowledgements
Members of the Koala Infectious Disease Research Group, Dr Richard Malick, Dr Sam Gilchrist (Sydney Wild Life World) and Australia Zoo Wildlife Hospital. This project is supported by the Hermon Slade Foundation and Boehringer Ingleheim.

References
INVESTIGATION INTO THE EFFECT OF DIFFERENT BAIL ACTIVATION SEQUENCES WITH A ROBOTIC ROTARY

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Second year, full time (Master of Science in Veterinary Science)

During 2009 and early 2010 FutureDairy has been testing a prototype, 16 bail, robotic rotary (Delaval Automatic Milking Rotary, AMR™). This Robotic Rotary (RR) is capable of carrying out in the order of 50 cow milkings per hour (system with 2 robots). It is recognized that challenges arise when pushing the upper limit of the capacity and when the system is operated at a low utilisation level. At this stage, the RR does not have any auto cleaning functions. A small herd increases the possibility that bails that have harvested milk since the previous wash then remain idle for a period of time resulting in an unsustainable increase in bacteria growth within the cups and milk lines. A potential solution to the challenge is to activate only a set number of bails after a system wash and have additional bails activated at set times until the next system wash, whilst idle bails are simultaneously deactivated.

The trial was conducted over a one month period (21 March to 21 April, 2011) at the EMAI site (Camden, New South Wales). There were 16 observation sessions conducted in four separate blocks. During the trial 160 dairy cows were managed and voluntary milked with a prototype RR. The effect of different bail activation sequences on the throughput capacity and animal behaviour was studied by activating 50% of the bails after a system wash, in four different sequences, with and without a feed reward on the platform.

There was no difference observed on the cow trafficking with the different bail configurations (P= 0.85) but the teaser feed created a significant impact (P= 0.001). There was also a significant interaction observed on the probability of an available bail being utilized between the availability of teaser feed and the number of cows in the pre-milking waiting yard (P= 0.004). The bail activation sequence did impact on the system level milk harvesting efficiency with consecutive bail activation resulting in more robot operations being conducted simultaneously and more milk harvested per minute of operation time (P= 0.036).

Acknowledgements
This work was carried out within the FutureDairy program. We wish to acknowledge the investors Dairy Australia, DeLaval, NSW Department of Primary Industries and The University of Sydney. We would also like to thank Mikael Karttunen for his technical support of the RR, the FutureDairy team for their assistance and Dr Navneet Dhand for statistical assistance.
INVESTIGATION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II (MHC-II) DAB AND DBB DIVERSITY IN FREE-RANGING KOALA POPULATIONS ACROSS AUSTRALIA
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Final Year, full time.

Introduction
Microsatellite studies have suggested that koalas from southern Australia show low genetic diversity when they are compared with those from New South Wales (NSW) and South-eastern Queensland¹,², providing insights into their genetic diversity and population dynamics. However, pathogenic challenges (e.g. chlamydiosis and koala endogenous retrovirus) makes further understanding of the genetic diversity of the immune response, in particular the Major Histocompatibility Complex (MHC), relevant. Here we study two MHC class II genes in koalas from across eastern Australia in order to provide preliminary information on the genetic diversity and distribution of alleles/variants across populations.

Materials and Methods
DNA was extracted and sourced through collaboration from 187 koalas representing 12 populations from South-eastern Queensland, NSW, and a number of Victorian populations including from French Island and South Gippsland. The exon 2 of MHC class II DAB and DBB genes was amplified using PCR. MHC variants/alleles were sequenced and confirmed in all of the individuals using three approaches, direct sequencing of PCR products, sequencing of clone inserts and one stranded conformation polymorphism.

Results and Discussion
In this study we have identified a total of 10 DAB and 7 DBB alleles. Koalas from Queensland and northern NSW had the highest average number of DAB and DBB alleles per individual. All koalas from Victoria were monomorphic at the DBB gene and had low DAB diversity except for koalas from South Gippsland. These show similar patterns to studies looking at neutral markers in the same or similar koala populations. Koalas from Victoria, with low MHC-II diversity, could be more susceptible to infectious diseases like chlamydiosis. Conversely, the higher MHC-II diversity in northern populations could be driven by the co-evolution of MHC-II with the emerging Koala retrovirus (KoRV).

Acknowledgements
Jade Patterson, Tristan Lee, David Phalen, Sarah Jobbins for donation of samples.

References
FORMAL AND INFORMAL PIG MOVEMENTS ACROSS EASTERN INDONESIA – RISK OF CLASSICAL SWINE FEVER TRANSMISSION

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Introduction
Nusa Tenggara Timur province, located in eastern Indonesia, has the largest pig population in Indonesia with 85% of families owning at least one pig¹. Pigs are important in NTT being used for traditional ceremonies and supplementary household income for families. Classical swine fever was first confirmed in NTT province in 1998². This is a highly contagious disease that results in substantial economic loss with high mortality rates.

Materials and Methods
To investigate pig movements two studies were conducted on West Timor, Flores and Sumba islands. These included a cross-sectional study in nine markets with pig sellers and pig buyers and a survey with smallholder pig farmers in 18 villages. Questionnaire data was obtained from 2009-2010. Location information for pig source and destination premises was obtained along with drivers for pig movement. A Social Network Analysis was then conducted with this data to analyse pig movement networks for the province.

Results and Discussion
Following interviews with 862 respondents (292 sellers; 281 buyers; 289 farmers), both across and between island movement was detected. A total of 359 villages were reported in the network. The markets identified with the greatest risk of spreading CSF virus through the network were Detusoko Market in Flores and Weta Bula Market in Sumba. Geographical features of the islands resulted in regional movement trends. Fragmentation was identified within the informal Flores network between the western and eastern regions resulting from a mountainous landscape with volcanoes and dense forest.

Acknowledgments
The authors would like to kindly thank the Australian Centre for International Agricultural Research (ACIAR), Pork CRC, Rob Christley, Simon Firestone, Mark Stevenson, Interview teams from Nusa Cendana University (West Timor), Yaspem Maumere (Flores) and Yayasan Cendana Mekar (Sumba) and staff members from Dinas Peternakan, Kupang.

References
CHARACTERISATION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE CANE TOAD, RHINELLA MARINA

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The invasive species, the cane toad (Rhinella marina), has experienced explosive population expansion following the introduction in 1935. Introduced as a biocontrol agent of agricultural pests, the cane toad population has expanded westward across northern Australia, and southward down the eastern coast. The cane toad invasion has had notable negative impacts on native Australian species through competition, predation and toxicity to predators of the toads. But the invasion process has also negatively impacted the cane toads themselves. Studies have reported physiological adaptations in the cane toad due to the invasion process, whereby cane toads on the invasion front are both physically and behaviourally more suited to dispersal, but also more susceptible to bacterial and parasitic infections (Brown et al. 2007). In order to investigation the impact of the invasion process on the cane toad immune system, we are using the adaptive immunogenetic marker, the major histocompatibility complex (MHC). This large gene complex is present in all jawed vertebrates with a key role in immune surveillance and response. The antigen presenting domains of the class I and class II genes are usually highly polymorphic due to positive selection via pathogen pressure and mate choice (Edwards & Hedrick 1998; Piertney & Oliver 2006). We have characterised the MHC Class I of the cane toad and are investigating the variation present in the northern cane toad population.

MICROBIAL PHYTASE INFLUENCES STARCH-PROTEIN DIGESTION KINETICS IN BROILER CHICKENS

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Though it is clear that microbial phytase improves the retention of phosphorus and calcium, the effects on protein and energy metabolism are equivocal. The aim of this study was to explore the effects of phytase on starch and protein digestion kinetics. In a 21-day deep litter trial, one-day old male broilers (n=840) were offered diets based on wheat and soybean meal (8.5 g/kg Ca and 3.25 g/kg nonphytate-P) and four phytase inclusion levels (0, 500, 1000 and 2000 FTU/kg). The diets were steam-pelleted at ~85°C and each dietary treatment was fed to 6 pens (35 birds/pen) Body weights, feed intake and mortalities were recorded. On day 21, five birds from each pen were euthanized and the proximal jejunum, proximal ileum and distal ileum were excised and digesta collected for assessment of starch and nitrogen digestibility. Starch and protein apparent digestibility coefficients at proximal jejunum, proximal ileum and distal ileum were calculated and are shown in the Table.

Table Phytase inclusion effects on apparent digestibility coefficients ratio

<table>
<thead>
<tr>
<th>Phytase (FTU/kg)</th>
<th>Starch Digestibility coefficients</th>
<th>Protein Digestibility coefficients</th>
<th>Coefficients ratio (Starch/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal jejunum</td>
<td>Proximal ileum</td>
<td>Distal ileum</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>0.736 0.929 0.973</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>0.714 0.945 0.980</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>0.781 0.902 0.966</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.031 0.018 0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (P=)</td>
<td>0.355 0.348 0.639</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear (P=)</td>
<td>0.185 0.944 0.531</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means with different superscripts are significantly different (P<0.05)

There was no effect (P>0.05) of phytase inclusion on starch digestibility at any part of the small intestine and no consistent effect of phytase on protein digestibility (Table). However, increasing phytase dose from 500 FTU to 2000 FTU resulted in improved synchronicity of recovery of starch and protein from the intestine. High phytase doses were associated with improved feed conversion ratios and superior weight gain (data not shown). It may be that part of these beneficial effects can be explained by a restoration, with higher doses, of synchronous absorption of amino acids and starch from the intestine. It can be concluded that high phytase doses may synchronize the net recovery of starch and protein from the lumen of broilers.

LIPID METABOLISM DURING PORCINE OOCYTE MATURATION AND EMBRYO DEVELOPMENT

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First year, full time

Introduction
Porcine oocytes have a greater lipid content compared to other mammalian species. It is believed that intracellular triglycerides play a metabolic role as an endogenous energy substrate during oocyte maturation, with phospholipids and cholesterol also a requirement for formation of membranes following cell divisions after fertilisation. However, the precise reason for the heightened lipid content in porcine oocytes is unknown. Increased intracellular lipid content leads to greater susceptibility to oxidative stress and increased sensitivity to low temperatures, limiting the application of cryopreservation of porcine oocytes and embryos. With pigs being a valuable resource in the livestock and biomedical research sectors, the ability to successfully cryopreserve large numbers of viable oocytes would be of significant importance.

This project aims to clarify the role of lipids during porcine oocyte maturation and embryo development, further to developing and refining methods of delipidation to allow cryopreservation of viable oocytes and embryos.

Materials and Methods
Initial work will look to quantify the number and distribution of lipid droplets within oocytes throughout maturation. This will involve staining oocytes using Nile Red, a fluorescent stain specific for neutral intracellular lipids, at several time points throughout in vitro maturation. Differences in lipid droplet number, size and distribution between oocytes of differing quality will be examined to determine if there is an association between lipid content and developmental competence.

Acknowledgements
Associate Professor Filip Braet, Dr Minh Huynh and Mrs Eleanor Kable of the Australian Centre for Microscopy and Microanalysis, The University of Sydney, for scientific and technical assistance with fluorescent staining and microscopy analysis and use of facilities; Wollondilly Abattoir for an endless supply of pig ovaries.

References
Cows managed in pasture-based automatic milking systems, usually show a lower milking frequency and total daily milk yield/cow, than those in indoor loose housing systems. In addition the proportion of milkings occurring after extended intervals, can be significantly higher which in turn impacts negatively on milk yield.

As a possible way to address this issue, a trial was conducted at the AMS farm in Camden, in late November – early December 2010, to evaluate the impact of 2 grazing management options on the occurrence of extended milking intervals and overall cow and system performance. For this purpose, two treatments (2 [2WG] vs. 3 [3WG] allocations of feed per 24h period), were compared as a pilot trial. The trial involved the entire milking herd of 158 cows, with average days in milk = 135 ± 8 days, average milking frequency = 1.49 ± 0.03 milkings/day, and average 7-day milk production = 20.5 ± 0.6 Kgs/c.d (Mean ± SEM). Cows were milked using 2 DeLaval VMS milking units.

Results indicated that cows in the 3WG treatment had a lower incidence of extended milking intervals, a 29% lower average milking interval, a 42% higher average milking frequency and a 20% higher average milk production. The increase in milking frequency and milk production for 3WG also resulted in a higher level of utilisation of the milking units during the day.

Results presented here are encouraging and support the message that farmers installing AMS should try to allow for capability to incorporate three way grazing due to the additional flexibility that it provides with managing extremely long (and short) milking intervals.

The authors would like to acknowledge the support of all sponsors of the FutureDairy project, particularly Dairy Australia, NSW Department of Primary Industries, The University of Sydney, and DeLaval. We would also like to thank the farm staff and technicians for their assistance.
A RETROSPECTIVE STUDY INTO THE ASSOCIATION OF MATERNAL PERICONCEPTIONAL NEGATIVE ENERGY BALANCE ON PROGENY REPRODUCTIVE PERFORMANCE: THE DAIRY COW PARADIGM

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Abstract
This research was originally presented at the 2011 COST GEMINI workshop in Israel\(^1\). Developmental programming is likely to have profound implications for the efficiency of livestock production. Given the decreasing reproductive performance (fertility being the top reason for low survivability), with selection for high milk yield and the homogenous nature of the genetics of Holsteins, it is thought that an environmental factor (maternal metabolic stress at conception) may be involved. During early lactation dairy cows usually experience Negative Energy Balance (NEB), especially in pasture based systems where nutritional intake can be limited or of lessened quality. This paper will present the influence of the dam’s energy balance at conception on offspring reproductive success. It is hypothesised that animals that are inseminated during periods of NEB will produce offspring who will have decreased reproductive performance.

Of the Holstein heifers recorded in this pasture fed data set, Dams of offspring were stratified for NEB using production variables including Days in Milk (0-90 or >90), Total and Peak Yield, Milk Protein at conception and Fat to Protein Ratio at mating. The relationship between estimated Energy Balance and Offspring reproductive performance (30 day InCalf Rate (ICR), 60 day ICR and Percentage Not Pregnant at mating end) was studied.

Preliminary evidence demonstrates that NEB has a significant association with offspring performance.

This study highlights the need for tightly controlled studies where dam’s Energy Balance is accurately measured.

Further studies are required to establish the precise mechanisms of action by which dam’s periconceptional metabolic stress affect progeny reproductive performance and specifically the duration and the intensity of NEB at mating on foetal programming of the reproductive axis in Holsteins.

References
HOOKWORM INFECTION IN THE AUSTRALIAN SEA LION, *NEOPHOC A CINEREA*

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Second year, full time

The Australian sea lion, *Neophoca cinerea*, is an endangered pinniped species endemic to Australian waters. The declining population consists of fewer than 15,000 individuals². Whilst fisheries by-catch and entanglement have been identified as significant threatening processes contributing towards the Australian sea lion’s decline², the contributory role of disease has yet to be elucidated. High pup mortality has been recorded in this species¹; 4; 5 and based on gross necropsies, the main reported causes are starvation and conspecific trauma³; 4. However, the cause of death is not always obvious and the numbers showing no detectable lesions can be as high as 71%⁴.

Our investigations at two of the largest colonies, Seal Bay and Dangerous Reef, indicate a high prevalence of hookworm infection in young pups and large burdens have been identified in dead pups. Consequently, we hypothesise that hookworm infection is a significant cause of clinical disease in Australian sea lion pups, leading to reduced growth rates and haemorrhagic enteritis and anaemia, subsequently increasing mortality rates directly or indirectly through increasing susceptibility to trauma and other disease processes. In order to quantify clinical effects and determine the degree of mortality attributable to hookworm infection in Australian sea lions, we are comparing the survival, growth, and haematological parameters of anthelmintic-treated and control (saline-treated) pups. These results will also be used to assess the potential benefits, limitations, and contraindications of employing anthelmintic treatment as an interventional management tool to facilitate the recovery of endangered populations.

References


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BREED ANALYSIS MAY REVEAL THE GENETIC COMPONENT OF CANINE ATOPIC DERMATITIS

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Third year, full time.

Atopic dermatitis is a complex disease which has environmental and genetic components in both humans and animals. Canine atopic dermatitis (CAD) occurs in at least 100 dog breeds, although some are only sporadic cases. Most canine studies report highly represented breeds without comparison to a base general dog population. Genomic and candidate gene approaches to CAD are reported, but the molecular basis of CAD is still unknown. We hypothesize that the genetic component is likely to be stronger in dog breeds with high relative risk and a common genetic origin. We aim to determine dog breeds which are susceptible to develop CAD in multiple countries, have high relative risk and a common genetic origin.

A meta-analysis was performed of eleven controlled CAD prevalence studies from 1971 to 2000, which used several statistical tests to compare the highly represented atopic dog breeds to a base general dog population. A ratio index was used to identify dog breeds globally susceptible to develop CAD.

The computerized clinical files of 852 confirmed CAD cases from over 23,000 dogs attending the two Sydney University Teaching Hospitals between the years 2001-2009 were reviewed and breed relative risk in both locations were calculated. Fourteen dog breeds were identified to have relative risk higher than 1.5. These breeds were analysed using a genetic cladogram¹ and compared to highly represented breeds identified in the meta-analysis.

The meta-analysis, prevalence study and the cladogram analysis suggest a common genetic origin for many of the dog breeds that are susceptible to CAD. Furthermore, one highly represented group of breeds is identified, which contains several high relative risk breeds from this study as well as breeds highly represented globally. Ongoing research includes an analysis of functional genomic and immunological studies, focusing on atopic cases from breeds belonging to this highly represented dog group.

Acknowledgements


References

WHEN DID TASMANIAN DEVILS LOSE MHC DIVERSITY?

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²The Australia Centre for Ancient DNA, University of Adelaide, SA

Third Year PhD, Full-Time

Abstract

The Tasmanian devil (Sarcophilus harrisii) is at risk of extinction due to the emergence of a contagious disease known as Devil Facial Tumour Disease (DFTD). This disease is a highly unusual transmissible cancer which is transferred between individuals by biting and does not invoke an immune response. The emergence and spread of this disease has been linked to a lack of diversity in the Major Histocompatibility Complex (MHC) genes of the devil. Tasmanian devils have survived several population crashes in the last two centuries which may have caused a loss of MHC diversity. Alternatively, MHC diversity may have been lost prior to European colonisation. The aim of this study was to determine when Tasmanian devils lost MHC diversity. To determine this we have cloned and sequenced the class I MHC alleles from historical Tasmanian devil samples. This includes samples collected at several time points post European colonisation (<1875 to today) during which devil population fluctuations were occurring, as well as samples from prior to European colonisation (both Tasmanian and mainland Australia). No additional diversity was seen in Tasmanian devils prior to the population crash time points indicating that these events did not significantly contribute to a decline in MHC diversity. A few additional alleles were seen in ancient mainland devils but these are highly similar to modern alleles suggesting that low MHC diversity has been present in the devil population prior to the extinction of devils on the mainland.
ASSESSMENT OF THE RISK FOR RABIES INTRODUCTION AND ESTABLISHMENT IN LOMBOK, INDONESIA

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¹Veterinary Faculty, University of Sydney, ²BBVet Denpasar, Bali

First Year Master Research (full time)

Abstract

Lombok Island is currently and historically a rabies free island in eastern Indonesia¹. However it faces the threat of rabies introduction from rabies infected, neighboring islands; Flores to the east, infected for 14 years and Bali 35 km to the northwest with island wide notification of human rabies cases since introduction in late 2008. Illegal importation of dogs from these rabies infected islands is a pathway to introduce rabies into Lombok, due to high number of fishermen and of people travelling by ferries. Indonesian fishermen have a cultural belief that bringing a dog with them while they sail, will make their journey safer related to the fact that animals have instinct to sense coming natural events such as earthquakes and storms¹. In addition to assessing pathways for rabies introduction, data on the size and structure of the dog population in Lombok is needed to determine the likelihood of exposure and spread of rabies among dogs on this island. Therefore, this study aims to obtain data on the size and structure of the dog population on Lombok Island, to determine pathways for release, exposure and spread of rabies virus among the dog population on Lombok Island, to assess the overall likelihood of introduction and establishment based on these pathways and also to evaluate the impact of alternate mitigation strategies on the overall likelihood. Scenario trees showing likely pathways of release, exposure and spread will be determined and then parameterized using information drawn from the literature, expert opinion and research on the dog population in Bali and Lombok. This quantitative risk assessment model will be evaluated to identify the parameters that most influence the overall likelihood and establishment. This study will have provide the relevant government authorities in Lombok and neighbouring islands with recommendations on quarantine and surveillance activities to reduce the transmission of rabies virus among dogs on Lombok in the event of a rabies incursion.

References

INVESTIGATING DISEASE SPREAD IN WILDLIFE: A STUDY OF SALMONELLA IN FERAL PIGS (SUS SCROFA)

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3 The School of Biological, Earth & Environmental Sciences, UNSW, Sydney, NSW.
4 Primary Industries NSW, Elizabeth Macarthur Agricultural Institute, Camden, NSW.
5 Institute for Applied Ecology, University of Canberra, Canberra, ACT.
6 Vertebrate Pest Research Section, DAF, WA, Forrestfield, WA.

Third year, full time.
Current understanding of the dynamics and drivers of disease spread in wildlife populations is limited, despite their involvement in many recent significant disease outbreaks worldwide, eg Sudden Acute Respiratory Syndrome, Classical Swine Fever, Foot and Mouth Disease (FMD). Feral pigs are a widespread wildlife species constituting a major biosecurity threat1. This study investigates endemic disease spread, Salmonella, in feral pigs in the Kimberley, Western Australia, by combining molecular epidemiological methods with demographic, geospatial and remotely sensed environmental data to examine the presence and risk factors for disease. It is part of a wider project which aims to improve understanding of emergency disease dynamics in wildlife by augmenting this study’s findings with information on the feral pig population structure from genetic analyses and details of population distribution based on aerial surveys. Similar studies of Salmonella in sympatric cattle in this region will also be undertaken. This data will be used to inform and parameterise computer simulation models of FMD, to help determine the potential role of feral pigs in an outbreak and to test appropriate surveillance and control strategies. In this study helicopters were used to sample 651 feral pigs at geo-referenced locations in a 20,000 km2 study area. Demographic data were collected for all pigs. Mesenteric lymph nodes (MLNs) and faeces were taken for Salmonella culture and serotyping. Salmonella was cultured from a total of 240 animals (37% (95%CI 33.2-40.6%). Overall 44 different serovars were isolated from faecal and MLN samples, 17 of which (39%) were found in both samples. The most commonly isolated serovar was S. Anatum (20% of faecal and 16% of MLN isolates). Salmonella serovars are being further characterised using pulsed field gel electrophoresis2. Analysis of risk factors for Salmonella infection are also being performed and a detailed spatial analysis of serovars will be undertaken to understand Salmonella transmission patterns in this population. Preliminary results and conclusions of this work will be presented.

References
THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) OF THE BLACK FLYING FOX (PTEROPUS ALECTO)

Justin Ng¹,², Katherine Belov¹, Lin-Fa Wang² and Michelle Baker²

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² CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong.

Abstract

MHC class I molecules play an important role in the immune response to viral infection, presenting endogenously derived peptides to cytotoxic T cells¹. Bats in particular, have been widely recognized as reservoir hosts to numerous high profile viruses²,³, with the deadly Hendra virus being found in Pteropus alecto in Australia⁴. Therefore, it is paramount to examine the diversity and repertoire of P. alecto’s MHC region. Bioinformatics tools were used to interrogate a closely related species’, P. vampyrus, genome publicly available in Ensembl. Primers were then designed to amplify full length MHC class I genes from P. alecto thymus cDNA. To date, 7 MHC class I genes were identified. Phylogenetic analysis revealed that all 7 class I genes clustered in a species specific manner as expected. Characterisation of four selected P. alecto class I genes across various tissues was completed using real-time PCR revealing a ubiquitous expression pattern, a characteristic typically associated with classical class I genes. This poses a possibility of bats having a larger / more diverse repertoire of classical class I genes than we humans do. With the aid of a P. alecto BAC library, mapping of the MHC class I region is in progress, thus further clarifying the MHC diversity. Investigation into polymorphism of the peptide binding groove is also currently underway. Through this project, we hope to identify unique / novel aspect(s) of the bat class I MHC region and understand its amazing ability to coexist asymptomatically with deadly viruses.

References

ARE VERTEBRATE PESTS A DISEASE RISK FOR COMMERCIAL PIGGERIES?

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2 Invasive Animal Cooperative Research Centre, South Australia
3 Australian Pork Limited, Deakin, ACT

Fifth year, part time.

Introduction
Understanding interactions between vertebrate pest species and domestic pigs, and the potential for transmission of pathogens, is essential to reduce risk of disease introduction into commercial piggeries and improve on-farm biosecurity practices. Historical examples of disease transmission from pest animals to domestic pigs include many classical swine fever outbreaks in the pig industry which have resulted from domestic and wild pig interactions and co-infections. The classical swine fever epidemic among domestic pigs in the Netherlands during 1997/1998 cost an estimated US $2.3 billion. Such interactions and their role in emergent diseases have the potential for major impacts on the pig industry including severe economic losses. This study is an assessment of the risk of introduction of pathogens from vertebrate pests into commercial piggeries in Australia.

Materials and Methods
We conducted a nationwide survey of commercial pig producers in 2007 to identify the wild and feral animal species commonly seen on piggeries in Australia. Species commonly recognised as vertebrate pest species in this country and identified as frequenting piggeries in high numbers or on a regular basis in this survey were European starlings (*Sturnus vulgarus*), rats (*Rattus rattus* and *Rattus norvegicus*) and feral pigs (*Sus scrofa*). These three pest species were subsequently targeted in separate studies, to identify specific pathogens of importance to the pig industry that could potentially be transmitted from these species to domestic pigs. For each pest species we conducted on-farm investigations during 2008 to 2010 targeting relevant pathogens.

Results and Discussion
1. European starlings were sampled for Salmonella, *Escherichia coli*, Campylobacter, Avian Influenza, West Nile Virus and Newcastle’s Disease Virus;
2. Rodents were sampled for *Brachyspira hyodysenteriae*, Salmonella, *Lawsonia intracellularis* and *Brachyspira pilosicoli*;
3. Feral pigs were sampled for Leptospirosis, Brucellosis, *Lawsonia intracellularis*, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*. Moreover, seven pigs were collared with GPS tracking devices to determine the length of time feral pigs were within varying distances from piggery facilities.

The risk of exposure was quantitatively assessed using the OIE framework for import risk analysis. This exposure assessment describes the pathways required for domestic pigs to become exposed to the relevant pathogens.

Acknowledgements
Supported in part by Australian Pork Limited, Invasive animals CRC, Victorian Department of Primary Industries, Wildlife Health Network and the Queensland Murray Darling Committee.

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56
GENOMIC ORGANISATION AND EVOLUTION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX OF SUIDAE AND TAYASSUIDAE

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³Animal Breeding and Genomics Centre, Wageningen University, the Netherlands.

First year, full time

Introduction

The Major Histocompatibility Complex (MHC) is one of the most gene dense and dynamic regions of mammalian genomes and plays a key role in immunity and response to pathogens. The MHC spans 2 to 3 megabases in mammals and comprises gene families involved in the antigen presentation to T cells. The MHC is a model genomic region to study evolution of histocompatibility gene families, and co-evolution between host and pathogen. MHC studies have mostly focused on species from major lineages. For the suids (Suidae), the MHC of the domestic pig (Sus scrofa) has been extensively sequenced and annotated¹. Our aim is to enlarge MHC studies to other suids as well as to its sister family tayassuids (Tayassuidae) by combining previous knowledge on S. scrofa and mammalian MHCs with new resequencing approaches. Indeed, suids and tayassuids are of particular interest since they represent both livestock and wild animals with specific issues related to zoonotic diseases and disease susceptibility in the wild. In addition, MHC data from suids and tayassuids will provide insights into the mechanisms of evolution that have been shaping the diversity of this genomic region since both families diverged from the common ancestor ~35-39 million years ago².

This research aims:
- To assemble and annotate the MHC genomic region for several species of suids and tayassuids using data generated by two methods; whole genome sequencing and heterologous capture of genomic regions for targeted sequencing.
- To investigate the phylogenomic relationships and mechanisms of evolution and selection that have shaped the MHC diversity among species of suids and tayassuids.
- To assess the genetic diversity of the MHC within species using two representative tayassuid and suid species.

Methods

We have used heterologous capture followed by sequencing and whole genome Next Generation Sequencing to generate a large MHC suid and tayassuid dataset representing ~140 individuals from 13 species. This dataset will be subject to quality controls, local and global assembly, alignment and annotation using the existing S. scrofa MHC reference sequence along with those from other mammals. The accuracy of the above assembly will be validated by selecting random contigs followed by primers designed for PCR and sequencing. MHC sequence drafts will be used for phylogenomic and selection analyses across species. Population genetic tools will be used for analysis within species.

References

A MONOGENIC CANDIDATE TRAIT APPROACH TO VALIDATE HIGH-RESOLUTION COMPOSITE SIGNALS OF SELECTION IN THE CATTLE GENOME

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Introduction

Phenotypic variation is in part attributed to underlying genetic effects and variation in the genome. The process of adaptation, domestication or commercial utilization of species results in strong selection for or against specific traits (phenotypes); therefore, a signal of such selection should be evident in the underlying genetic regions1. Theoretically, the monogenic traits with high penetration (heritability) are expected to result in strong divergent selection signatures. Typically such selection signatures can be detected with various methods analysing in part different aspects related to selection signatures (see below). A combination of the output from different methods could also increase the resolution of selection signals many fold2. Here we report the investigation on two monogenic traits (polledness and double muscling) which have prominent phenotypic characterization in certain cattle breeds.

Materials and Methods

In total, 38,610 SNPs genotyped using the Illumina BovineSNP50 chip assay on 375 animals of 21 beef cattle breeds were used. Independent analyses were carried out on two data subsets, each containing various breeds grouped for the presence or absence of either polledness or double muscling. Various test statistics were calculated including population differentiation (FST/di), across population extended haplotype homozygosity (XP-EHH), change in derived allele frequency (ΔDAF) and integrated haplotype homozygosity score (iHS). To test the existence of a common selection signature, a composite signal at each SNP was computed as the mean Z scores of multiple methods obtained by converting the fractional ranks of test statistics of each method into Z-statistics and summed in a composite score.

Results and Discussion

Genome-wide distribution of the composite signals found the highest scores for polledness and double muscling on bovine autosome (BTA) one and two, respectively. The candidate regions on BTA-1 and BTA-2 harbour the functional mutations in genes, synaptojanin 1 (SYNJ1)3 and myostatin (GDF8)4, causing polledness and double muscling in cattle, respectively. The existence of strong signals close to the candidate genes, even in the absence of any casual SNP in the genotype data, confirms the robustness of the breed grouping strategy and methodology for deriving composite signals. This approach could help, doing analyses based on the specific cattle breed groups for their complex phenotypes, identify the candidate regions especially carrying genes involved in domestication, adaptation and production traits.

References

IDENTIFYING RISK FACTORS OF LAMENESS IN DAIRY HERDS ACROSS NSW

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Third Year, Full Time PhD

Introduction
Lameness is considered the most important welfare concern for dairy cattle[1]. It is also
among the three major causes of involuntary culling of dairy cows after infertility and
mastitis. Prevalence of lameness has been analyzed in several investigations, ranging
between 12.8 to 24.6%[2]. Lameness has been associated with various risk factors such
as season[3], parity[2, 4], and walking on poorly maintained tracks[5]. The prevalence of
lameness has not been yet studied in NSW. One of the objectives of this research is to
assess farms across NSW for potential risk factors for lameness. The prevalence of
lameness and the observations on each farm will be correlated to address the potential
risk factors for these farms. This information will assist the producers and farm advisors
to promote adoption of facilities and management practices that minimize the incidence
of lameness.

Materials and Methods
A total of 100 dairy farms will be participating in the study. An investigation protocol has
been developed. Each farm is visited at least once (some twice depending on the herd
size) over the duration of the study. During the visit the herd is observed while walking
towards the holding yard. After milking the whole milking herd is locomotion scored
using a 4 point method (developed by Cook[6]). The farm environment is then assessed
by the investigator according to the protocol. The farmer is then interviewed about herd
management practices used on his farm.

Results and Discussion
A pilot study was performed in order to validate the investigated areas and questions.
So far the results show an average prevalence of 30% ranging from 38-23%. The data
on risk factors will be analyzed at the end of the project.

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CLINICAL AND FINANCIAL IMPACT OF TOXOCARA VITULORUM AND FASCIOLA GIGANTICA IN CATTLE AND BUFFALO IN NORTHERN LAO
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Introduction
In many parts of Asia Toxocara vitulorum and Fasciola gigantica infections in ruminants are endemic and contribute to low productivity ¹, ². In northern Lao both parasites are considered endemic and a major production constraint based on anecdotal reports. Better control of these parasites is expected to increase large ruminant productivity and assist smallholder farmers to move out of subsistence farming. This research aims to assess the financial and clinical impacts of these parasites as well as farmers’ knowledge, attitudes and practices towards parasite control in order to provide recommendations on cost-effective and context appropriate approaches to control.

Materials and Methods
Between September 2009 and February 2011 prevalence studies using faecal egg counts (FEC), followed by farmer interviews to collect data on calf morbidity and mortality, large ruminant productivity, household finances, farmer knowledge, attitudes and practices were completed. Two-stage sampling of villages and then households was undertaken in 5 northern Lao provinces. Total animals sampled were 886 cattle and buffalo calves < 3 months old from 65 villages for T. vitulorum and 1270 cattle and buffalo >12 months old from 75 villages for F. gigantica. Around 250 owners of the animals sampled for each parasite were interviewed 3-7 months after sample collection. An abattoir survey at 5 provincial slaughterhouses to assess carcass damage was completed from March to June 2011 and 69 buffalo and 56 cattle were assessed. Treatment trials for both parasites and genetic analysis of fluke specimens are in progress.

Results
T. vitulorum prevalence: 76% of the villages had FEC positive calves. 21% of cattle and 26% of buffalo calves were positive. F. gigantica prevalence: 73% of villages had FEC positive animals. 15% of cattle and 22% of buffalo were positive. At slaughter 96% of buffalo and 38% of cattle had gross liver lesions consistent with fluke infection. Of the animals with liver lesions 36% of buffalo and 30% of cattle were FEC positive. Abnormal livers or other products were not condemned and were sold at local meat markets for the same unit price as normal products.

Discussion
Results confirmed that the prevalence of both parasites is high in northern Lao. The slaughterhouse survey indicates that FEC underestimates the true prevalence for F. gigantica. The lack of meat inspections, condemnation and current non-discriminatory market demand for large ruminant meat products provide a potential disincentive for farmers to control parasites. Initial results from farmer interviews indicate that farmer knowledge is low and any effective management of either parasite is absent. Full analysis of the interviews and results of treatment trials are hoped to provide evidence for practical and acceptable control options for T. vitulorum and F. gigantica for Lao smallholder farmers.

Acknowledgements
ACIAR and W Richards Award for their financial assistance for the research. Lao farmers and government staff for assistance with field work. Sonevilay Nampanya and Bounthom Khounsy for Lao-English translation work and field work assistance.

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PROTEOMIC ANALYSIS OF RAM SEMINAL PLASMA

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Introduction

Despite considerable research in male reproductive physiology, mystery continues to surround the precise nature of seminal plasma and the role this substance plays in sperm physiology, function and fertility. The utility of frozen thawed ram spermatozoa is limited due to poor fertility following cervical artificial insemination1. There is evidence to suggest that seminal plasma proteins interact with the sperm membrane and are either negatively or positively affected during cryopreservation, impeding the ability of the spermatozoa to traverse the female tract2. However, relatively little is known about the identity of these proteins. Therefore, the current study aims to identify and characterise the major seminal plasma proteins of the ram.

Materials and Methods

Seminal plasma was obtained from 20 rams, ranging in age from 1-10 years and breed. Seminal plasma proteins from each ram were separated via 1D-SDS PAGE, reverse phase liquid chromatography (RPLC) and 2DLC- MS/MS, where RPLC was coupled to strong cationic exchange (SCX). Isolated proteins were then analysed by MS/MS (QSTAR® Elite; AB SCIEX, USA). Peptide spectra were then interpreted using Protein Pilot v3.0. (Applied Biosystems, USA) and further collated and refined using Scaffold v3.0 (Proteome Software Inc, USA). 1D -SDS PAGE was also used to investigate differences in the seminal plasma proteome of rams varying in sperm quality. Densitometry analysis was used to reveal potential differences in protein band concentration between rams varying in phenotype.

Results and Discussion

LC-MS/MS identified 356 total proteins across all three separation techniques. A total of 26 proteins were detected via 1D-SDS PAGE, 34 proteins by RPLC and 296 proteins from 2D LC-MS/MS. Collated and filtered, a total of 156 individual proteins were detected in the seminal plasma of the ram. The absence or presence of protein bands between rams of different breeds/sperm quality, indicate a difference in the proteome of rams of varying phenotypes. To our knowledge this is the first study to characterise ram seminal plasma and compare proteomes between individual rams varying in sperm quality. These results provide an important first step in understanding the nature of ram seminal plasma and how it interacts with spermatozoa, affecting fertility.

Acknowledgements

The authors would like to acknowledge the help of Dr Rosyln Bathgate, Dr Claire Kershaw-Young, Andrew Souter, Kim Heasman, and Byron Biffin.

References

REAL-TIME POLYMERASE CHAIN REACTION (PCR) DETECTION OF DWARF GOURAMI IRIDOVIRUS (DGIV), AN IMPORTANT EMERGING EXOTIC VIRAL PATHOGEN OF ORNAMENTAL FISH IN AUSTRALIA

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Introduction
The movement of ornamental fish through exporting and importing practices provides a transmission pathway for the introduction and establishment of exotic viral pathogens. The viral agent dwarf gourami iridovirus (DGIV) represents a significant exotic pathogen to Australia having been implicated as the cause of severe disease and mortality in intensively raised fish and ornamental fish. Previous studies determined that ornamental fish entering Australia may carry pathogens of quarantine concern, specifically DGIV. Murray cod (Maccullochella peelii peelii), in the Family Pericichthydae, is an iconic species of high conservation value in Australia which has been shown to be susceptible to DGIV¹. The disease threat to the aquaculture industry and native fish of Australia has driven the development of molecular diagnostic tests capable of detecting this virus.

Materials and Methods
Optimised real-time polymerase chain reaction (PCR) assays were employed in a survey of ornamental fish to determine whether DGIV is in fact entering Australia despite quarantine practices, and further, to determine whether this virus is already established in farmed or wild ornamental fish populations in Australia. A series of surveys of ornamental fish is being conducted to detect DGIV beginning with fish that have just arrived in Australia (pre-border), those in quarantine (in quarantine approved premises), fish just released from quarantine (post border), and ornamental fish already in Australia (in retail shops and farmed and wild fish).

Results and Discussion
The outcomes from this research have important implications for both quarantine policy and management of ornamental fish in Australia.

Acknowledgements
This research was funded by the University of Sydney and the Fisheries Research and Development Corporation (FRDC).

References
MECHANICAL PERFORMANCE OF A LOCKING PLATE WITH CYCLIC LOADS

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Background
Locking plates with threaded locking head screws were developed for biological fracture fixation. These implants act as internal fixators and do not require friction to be maintained between the plate and the bone for stability. The screws are angularly stable and so are more resilient to screw pullout and implant loosening. Therefore locking plates can be placed directly against the bone or slightly elevated from the underlying periosteum, preserving blood supply. A potential disadvantage of leaving a large gap under the plate though, is a reduction in fixation stability.

Objective
We hypothesized that the in vitro mechanical stability of a locking plate system that relies on a plate-screw conical coupling may be compromised when the plate is elevated 2 mm from the bone and placed under conditions of cyclic loading.

Materials and Methods
Paired femora (n=6 pair) were harvested from cadavers and 3.5 series 3mm thick 3-hole Fixin plate constructs were applied to the bone either with direct plate to bone contact or with a 2mm plate to bone gap. The constructs were cyclically loaded on a material testing machine in 10 percent incremental increases every 1000 cycles at 2 Hz with a range of 250 Newtons to >350 Newtons, fatiguing the constructs to failure.

Results and Conclusions
The mean sustained loads in the contact group (420.8 Newtons, 7612 cycles) were significantly greater than in the 2mm gap group (337.5 Newtons, 4252 cycles) (p-value <0.001). Failure mode of all constructs was via screw pullout (contact n=6, 2mm gap n=6). All of the plate-bone constructs failed by pullout of the screw closest to the osteotomy site. After mechanical testing, all fatigued constructs were embedded in polymethyl methacrylate, sectioned with a diamond saw and the screw-plate interfaces and screw-bone interfaces examined using a stereo zoom microscope (SZM) and scanning electron microscope (SEM). After examination under SEM, it was determined that the failures occurred as a breakdown of the bone between the screw threads, leading to screw pullout. Results suggest that elevating locking plates with a conical coupling system 2mm from the bone did not result in coupling failure but reduced overall construct fatigue life. However, further evaluation is required to determine if this is of clinical significance.
Devil Facial Tumour Disease (DFTD), a transmittable and fatal cancer has decimated the wild population of Tasmanian devils (Sarcophilus harrisii) to the point where they are now threatened with extinction and listed on the IUCN red list. This disease, which originated in the north east of Tasmania, has now spread throughout ~80% of the devil’s range. The cancer is caused by a clonal cell line which originated in a single devil and is spread from animal to animal by biting. Due to a paucity of genetic diversity, especially amongst the genes of the Major Histocompatibility Complex (MHC), the Tasmanian devil’s immune system does not recognise the tumour as non-self and does not mount an immune response.

The aim of this study is to investigate the role of MHC in mate choice within the Tasmanian devil insurance population, housed in zoos and conservation parks across Australia. As well as a key role in immune response, MHC genes are also thought to play an important role in mate choice with animals preferring to breed with mates that differ at their MHC to maximise the immunological fitness of their offspring. The hypothesis to be tested is whether breeding success correlates with MHC dissimilarity.

My research project will track microsatellite diversity at both neutral and MHC linked loci. Preliminary analyses show high levels of heterozygosity amongst the insurance population and no evidence of inbreeding. The next phase of the project will be to explore the role of MHC and mate choice within this captive insurance population.
STRAIGHT FROM THE HORSE’S MOUTH: HORSE MANAGERS’ RATINGS OF THE 2007 EQUINE INFLUENZA OUTBREAK MANAGEMENT

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Australia's first-ever outbreak of equine influenza in 2007 spread over a large geographic area but was contained and eradicated within five months as a result of a substantial disease control effort led by Federal and State animal health authorities. Despite its timely control, the outbreak and associated control measures caused severe disruption to horse owners and industry participants. This study conducted with New South Wales horse managers affected by the control measures aims to describe their perceptions of the outbreak management conducted by the State government’s animal health authority. Face-to-face interviews with 200 managers were conducted in 2009 and the ordinal ratings of their perception of outbreak management (‘well managed’, ‘adequately managed’, ‘poorly managed’) were analysed using ordinal logistic regression to elucidate factors associated with a ‘well managed’ rating. Of the managers interviewed, 40% regarded the outbreak ‘well managed’, 40% considered it ‘adequately managed’ and 20% thought the outbreak was ‘poorly managed’. The final multivariable logistic regression model was adjusted for age and gender of managers. Those respondents managing a horse stud were 11 times less likely to consider the outbreak ‘well managed’ than those who did not. Interviewees believing another outbreak of equine influenza was highly likely in the next five years were more than 3 times less likely to deem the outbreak response ‘well managed’ compared to those thinking it was unlikely or not at all likely. Those involved in horse competitions/sporting events were two times less likely to rate the outbreak ‘well managed’ compared to those who did not compete. Managers from certain regions were more likely to consider the outbreak ‘well managed’. These findings should be considered to promote horse manager-government rapport and future compliance with disease control regulations.

Acknowledgements:
This research was funded by the Rural Industries Research and Development Corporation (RIRDC). We thank the horse managers interviewed for their time and cooperation and the NSW DPI for making the equine influenza data set available.
UNDERSTANDING COW QUEUING BEHAVIOUR WITHIN THE NEW ROBOTIC ROTARY AUTOMATIC MILKING SYSTEM

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Introduction
Automatic milking systems (AMS) are relatively new to the Australian dairy industry, and present new and exciting challenges associated with pasture-based dairy operations. AMS facilitates voluntary milking. As a result, cows voluntarily wait in the pre-milking holding yard until they choose to be milked. Understanding queuing behaviour may enable the development of management strategies to minimise extended waiting times, increase system efficiencies, and reduce the incidence of cows spending extended periods of time off pasture and on concrete.

This study investigated the voluntary waiting time in the pre-milking holding yard of a herd of approx. 170 dairy cattle milked using an Automatic Milking Rotary (AMR™).

Materials and Methods
Feed on the platform was provided for half of the trial, with treatments “feed on” and “feed off”. The waiting time was calculated as the time from entry into the holding yard until the commencement of milking on the robotic rotary (RR).

Results and Discussion
The provision of feed on the RR significantly reduced the voluntary waiting time (average 76.4 min vs. 152.8 min for feed and no feed respectively), and increased the number of milkings by 56 per day. Older cows had significantly longer waiting times, and heifers were associated with the shortest voluntary waiting times, as expected and in agreement with findings reported in the literature.¹ Higher producing animals demonstrated significantly shorter waiting times, potentially due to a greater motivation to find fresh feed. It was concluded that the provision of feed on the RR is a valuable management strategy for reducing voluntary waiting time in the pre-milking holding yard. Future research should target those cows with extended waiting times to further understand why they voluntarily wait in the dairy for long periods of time prior to milking.

Acknowledgements
The investors of FutureDairy (Dairy Australia, NSW Department of Primary Industries, DeLaval and The University of Sydney) and the staff at the Camden AMS research farm.

References
SEROPREVALENCE OF COXIELLA BURNETII IN DOMESTICATED AND FERAL CATS IN EASTERN AUSTRALIA

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Coxiella burnetii (C. burnetii) is the bacterium responsible for Q fever, an extremely important worldwide zoonosis, notifiable within Australia¹ and classified by the Centers for Disease Control and Prevention as a category B biological terrorist agent². Infected domestic ruminants represent the most commonly reported sources of infection³, but over 50% of the reported cases in people in Australia have an unknown source of infection with their occupations being unlisted or unknown⁴. Other sources of infection require further investigation. While there are several reports of parturient cats as reservoirs for human infection⁵,⁶,⁷,⁸,⁹ the role of the domestic cat within households is unknown and largely unexplored. The seroprevalence of C. burnetii in different subpopulations of cats in Australia is of paramount importance to establish the risk that non-parturient and parturient cats pose to veterinary personnel and the cat owning community. The three main serological tests used in other species include; complement fixation test (CFT), indirect immunofluorescence assay (IFA) and enzyme linked immunosorbent assay (ELISA)¹⁰. We have developed an IFA method to determine the seroprevalence of C. burnetii in pet cats, feral cats, cattery-confined and shelter cats. Following two-fold serial dilutions from 1/2 to 1/1024, a cut off titre for phase I and II antibodies was determined to be 1/64. To date 10.6% (21 out of 198) of pets cats have antibodies to phase II antibody and 0.5% (1 out of 198) have antibodies to phase I and II antibodies. A comparative analysis of the three main serological assays will follow the seroprevalence study. Following recent outbreaks of Q fever in companion animal veterinary personnel in Sydney, there is an urgent need to elucidate the true seroepidemiological status of C. burnetii in domesticated and feral cats in Australia. The outcomes of this study may result in reforms in the current operating procedures in veterinary practices in Australia.

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BOLDNESS AS A PERSONALITY SUPER-Trait IN THE DOMESTIC DOG

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Introduction – Studying personalities in non-human animals may offer a framework to explain the variations in some behavioural responses and predict how individuals respond to certain stimuli. In many different species, an over-arching personality 'super-trait' has been identified. This is known as the shy-bold continuum \cite{1-3}. How this super-trait relates to the expression of behaviour has been examined previously in dogs \cite{4-7}. We followed up on this work by investigating variables that may affect the expression of boldness in dogs.

Methods – A survey was formulated using items from published studies \cite{5, 8, 9} to which we added items aimed at addressing proactive and reactive coping styles. The survey was circulated amongst dog owners via internet forums, e-mail lists, and social media. The results were explored using a principal components analysis, and further analysis was completed using linear regression models.

Results and Discussion – We obtained results from 1054 dog owners. One component was retained from the PCA, characterised by high loadings for items indicating boldness and negative loadings for items indicating shyness. It was thus labelled 'boldness'. Further analysis revealed that boldness scores are significantly higher in male dogs than female dogs, and significantly higher in entire dogs than desexed dogs. Age had a significant effect, with boldness decreasing with age. Breed and breed group also had a significant effect on boldness. Guardian breeds were the boldest group and companion breeds the most shy. Linking behaviour and other factors with boldness may arm dog owners with more information to help them select individuals that will best suit them, and be better prepared for the behaviour their dogs are likely to display.

References
REPRODUCTIVE PERFORMANCE OF DAIRY COWS IN AN AUTOMATIC MILKING SYSTEM

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Introduction
In automatic milking systems (AMS), the potential exists to increase milk production by about 5-10% if significantly higher milking frequencies (particularly in early lactation) are achieved (and the nutritional and physiological status of the cow is not limiting production). Increased milk yield can be associated with a more severe negative energy balance during early lactation¹. Moreover, active estrus detection in AMS farms may require a more conscious efforts as cows have different patterns of movement in AMS compared to conventional milking system in which estrus detection is generally carried out at milking time. Therefore, the aim of the study is to assess the reproductive performance of dairy cows at an AMS research farm.

Materials and Methods
A 5 year retrospective epidemiological survey will be conducted to assess the fertility of the dairy cows at an AMS research farm (Elizabeth Macarthur Agricultural Institute, Camden). The dataset will contain information about no. of lactation, milking frequency and milk yield, feeding, date for first insemination, date for calving, occurrence of postpartum diseases. From those data, the association between predictor variables (nutritional management, milk yield and its composition, body weight, BCS and disease occurrence) and outcome variables (calving interval, calving to first AI interval, calving to conception, number of AI per conception, days open, conception rate, pregnancy rate, cow survival rate and culling attributed to reproductive problems, lameness and mastitis) will be analyzed by regression model. Based on the findings of this analysis, succeeding trials will be designed. Data will be analyzed for experimental conclusion of each assay using Genstat version 13.

Acknowledgements
Future Dairy project, particularly Dairy Australia, NSW Department of Primary Industries, University of Sydney, DeLaval and International Postgraduate Research Scholarship.

References
GENERATION AND CHARACTERISATION OF CANINE INDUCED PLURIPOTENT STEM CELLS

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First Year PhD research (currently part time)

Abstract
Stem cells are undifferentiated, self renewing cells that have the capacity to differentiate into functional cells of various cell lineages¹. The potential of these cells to play a pivotal role in the treatment of many disease conditions in humans and animals has generated great interest in recent years. Stem cells may be obtained from the inner cell mass of a blastocyst, known as embryonic stem cells, or can be derived from adult tissues. Prior to 2007, adult derived stem cells were primarily obtained from bone marrow and adipose tissue. Although providing a readily available source of cells for clinical applications, these cells are considered multipotent in contrast to the pluripotent embryonic stem cells. In 2007, Takahashi et al² described the reprogramming of adult cells to express pluripotent transcription factors, reporting that these cells, termed induced pluripotent stem (iPS) cells, were identical to embryonic stems cells in morphology, proliferation, gene expression, in vitro differentiation and teratoma formation.

The initial stages of this project will involve reprogramming adult canine cells into iPS cells using non integrating mRNA reprogramming, therefore creating cells that could be theoretically utilised in a clinical setting. This technique has been used to generate human iPS cells³ but there are no current reports of the creation of canine iPS cells using this technique. The generation and characterisation of canine iPS cells will allow comparison to adipose derived mesenchymal stem cells (AD-MSC), which have been more extensively characterised and are currently being used in the treatment of canine osteoarthritis. Black et al⁴ has reported a clinical improvement in lameness following an intra-articular injection of AD-MSC in dogs suffering from osteoarthritis. No reports exist on the clinical application of canine iPS cells, however, it has been suggested that they may have future application in bone and tendon healing, cartilage repair and generation of models of disease processes.

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MICRORNA AND CANCER GENE EXPRESSION IN CANINE MAST CELL TUMOUR (MCT)

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2 year, PhD, full time

Introduction
There is growing recognition that annotation of tumour specimens with data integrating information about molecular alterations and gene expression provide a more complete understanding of tumour biology but also a significant opportunity for development of novel clinical tools and therapy. Studies have shown that mutations in the c-Kit gene are present in 9-30% of canine MCT cases¹ but the mechanisms of oncogenesis in the other 70% of MCT are yet to be elucidated. There is limited success in using standard tumour classification systems or presence of c-Kit mutations to identify subgroups of patients with the same diagnosis but different clinical and treatment response outcomes. This project will be the first to use RT²-qPCR array to comprehensively interrogate the gene expression profile of 96 canine and cancer-pathway specific genes in canine MCT. These include genes identified to be altered in expression profiles in human mastocytosis such as ETS1, ATM and CDKN1A.²,³ Abnormal patterns of miRNA expression have been associated with human cancers particularly the miR-17-92 cluster.⁴,⁵ This cluster is highly conserved with homologs in dogs and has recently been identified, amplified and characterized from normal canine tissues (canine autosome 22 region).⁶ Expression differences in miRNA profiles provide insights into the regulation of key genes important in tumour pathogenesis and expected to contribute to classifying tumours and predicting their outcomes.⁷,⁸ Molecular signatures will be correlated with clinical parameters allowing the stratification of tumours.

Objective
Development of an accurate predictive algorithm for the prognostication and treatment of canine MCT using molecular signature profiling

Materials and Methods
Patnaik grade 2 cutaneous MCT samples (n=14) and healthy skin controls (n=10) derived from various breeds were used for total RNA extraction using Qiagen® miRNeasy Kit. Quality control of extracted RNA was assessed by standard spectrophotometry and gel electrophoresis. RNA samples that passed quality assurance were converted to cDNA using Qiagen® First Strand Kit. Processed samples were subjected to RT²-qPCR (Rotor-gene 6000 Corbett) by aliquoting each sample with Qiagen® qPCR Fast Sybr Green/ ROX Master Mix into a Qiagen® Canine PCR array disc. Statistical analyses were performed using online PCR array data analysis tool www.SABiosciences.com/pcrarraydataanalysis.php. A portion of each sample was subjected to microRNA microarray profiling at the Ramaciotti Centre using the Affymetrix - Geneishpere FlashTag™ platform (Sanger miRNA database v12) and Genepattern™ software.
Acknowledgements

Neil & Allie Lesue Scholarship (2009); Goldia and Susie Lesue Scholarship (2010); Morris Animal Foundation Scholarship (2010); Sarah and Anne Payton Canine Cancer Research Grant (2010); Lionel Lonsdale Clinical Fellowship (2011)

References


HUMAN RABIES MORTALITY ESTIMATION IN RABIES ENDEMIC SOUTH BHUTAN USING DECISION TREE ANALYSIS

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Introduction
Dog bites in humans and rabies are a public health problem worldwide. Understanding the true scale of human deaths caused by rabies is important for public health planning program. In this study, we estimated the human deaths from rabies in two rabies endemic areas of south Bhutan and compare the estimates to the observed data.

Materials and methods
The distribution of dog bite injury on different body parts and the probability of developing rabies were used to estimate the human deaths from rabies using a decision tree model\(^1\). The decision tree model consists of 10 probability steps \((P1 \text{ to } P10)\). The first step \(P1\) is the rabies recognition probability in the dogs (the proportion of suspected rabid dog bites that are in fact rabid). For the \(P2\)–\(P5\) probability steps, the dog bite injury data were classified according to the distribution of the bites on the body: head/neck \((P2)\), hand/arms \((P3)\), trunk \((P4)\), legs/thigh \((P5)\), and age group of the victims: \(4\) (5–9; 10–14, and >15 years of age). The probability \((P6\)–\(P9)\) of developing rabies following the bite of a rabid dog to head \((P6)\), arms \((P7)\), trunk \((P8)\) and legs \((P9)\) were, 45\%, 28\%, 5\% and 5\% respectively (based on the published study)\(^1\). The probability of receiving post exposure treatment \((P10)\) following bite from a suspected rabid dog was determined on the basis of the previously published data (minimum=80\%; most likely=90\%; maximum=95\%)\(^2\). The probability of dying of rabies following a bite from a suspected rabid dog was determined on the basis of the previously published data (minimum=80\%; most likely=90\%; maximum=95\%)\(^2\). The probability of dying of rabies following a bite from a suspected rabid dog was calculated as: 

\[
\text{probability of death (Pdeath)} = P1 \times ((P2 \times P6) + (P3 \times P7) + (P4 \times P8) + (P5 \times P9)) \times (1-P10).
\]

Then the total number of deaths caused by rabies per year were calculated as: 

\[
Tdeath = (I \times Q \times \text{Pdeath/100,000}),
\]

where ‘\(I\)’ is the incidence of suspected rabid dogs bites per 100,000 population at risk per year and ‘\(Q\)’ is the total population at-risk \((n=47721)\) based on the 2005 population data of Bhutan. The confidence limits for the total number of deaths from rabies were calculated by assigning the probability distribution to the inputs parameters and running Monte Carlo simulations for 10,000 iterations using R software. The mean and the 95\% CI were recorded.

Results and discussion
The mean annual number of reported human deaths due to rabies (from 2006 to April 2011) in the two rabies endemic areas were 1.5 (95\% CI: 0.75–3.00), equivalent to an annual incidence of 3.14 (95\% CI: 1.57–6.29) deaths/100,000 population. Based on the dog bite survey data, the model predicted 2.23 (95\% CI: 1.20–3.59) deaths per year, equivalent to an annual incidence of 4.67 (95\% CI: 2.53–7.53) deaths/100,000 population. The annual predicted death were greater for ages <15 years compared to older age groups. In the absence of any post exposure treatment, the 223 suspected rabid dog bites would result in a total of 19.24 (95\% CI: 13.69–25.14) deaths per year in these two areas of Bhutan, equivalent to an annual incidence of 40.31 (95\% CI: 28.70–52.68) death/100,000. The sensitivity analysis of \(P1\) at different point estimates indicates that the predicted human rabies death increases with higher probability of rabies recognition. Predicted human deaths due to rabies from the decision tree model were almost the same as the annual mean human rabies deaths reported in these two areas, indicating that there is no serious under-reporting of rabies in Bhutan, although some extent of under reporting of dog bites would exists.

References
NOVEL GENE EXPRESSION PROFILE IN BLOOD CELLS FROM CATTLE EXPERIMENTALLY EXPOSED TO MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS
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3\textsuperscript{rd} year PhD, full time

Introduction
*Mycobacterium avium subspecies paratuberculosis* (Mptb) is the causative agent for the chronic debilitating condition called Johne’s (JD) disease affecting ruminants. Identifying and characterising the different strategies employed by *Mycobacterium avium subspecies paratuberculosis* (Mptb) to alter the host immune system in its favour and thereby persist intracellularly could hold the key to fighting this disease. Microarrays can be used to examine differences in the gene expression profiles of peripheral blood mononuclear cells during disease processes. In-vivo gene expression studies are crucial tools for uncovering immunopathogenic pathways of infection within an infected host.

Materials and Methods
Affymetrix Genechip Bovine Gene microarrays were performed using the RNA prepared from blood samples collected from 4 exposed and 4 non-exposed control cattle from the controlled experimental model of bovine JD (microarray experimental work and data analysis performed by Dr. Auriol Purdie). Bio-informatics tools and data from current literature were applied to identify interactive and parallel relationships between genes and physiological functions. Data Genes related to lipid pathways and anti-bacterial defence mechanisms were identified and validated in a larger cohort at 9, 12 and 21 weeks post-infection by quantitative polymerase chain reaction (qPCR).

Results
A list of seven genes comprising, CD38, NADH dehydrogenase (NDUFS3), GTP-ase immunity associated protein family member 6 (GIMAP6), low density lipoprotein receptor (LDLR)/ similar to low density lipoprotein receptor associated protein 11 (similar to LRP 11) (LOC617450), 24 dehydro cholesterol reductase (DHCR24), stearoyl CoA desaturase (SCD) and granulysin (GNLY) were selected for validation. qPCR validation confirmed the differential regulation of these genes in the Mptb exposed animals in comparison to the Mptb non-exposed animals.

Conclusion
A number of novel gene pathways were shown to be differentially regulated in bovine JD linking lipid pathways and anti-bacterial defence mechanisms. A model was developed that explores how these could impact the host immune response in a double edged sword manner that leads to the progression of pathogenesis.
MOLECULAR METHODS FOR THE DETECTION OF CALF SCOUR PATHOGENS

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Introduction
Neonatal calf diarrhoea (NCD) is a common problem in Australian beef and dairy enterprises. The disease epidemiology is complex, and traditionally diagnosis is difficult. Prompt, accurate and cost-effective diagnosis is imperative so our research group is exploring the potential application of real-time polymerase chain reaction (qPCR) and loop mediated isothermal amplification (LAMP) for faecal and environmental samples, as tools to add to the diagnostic armoury for this disease.

Materials and Methods
Initially we explored best means of faecal sample handling and storage followed by nucleic acid (both RNA and DNA) extraction from faecal samples, using a range of protocols. Magnetic beads and an automated magnetic particle handling system are being utilized. We then proceeded to develop and validate singleplex qRT-PCR assays for the viruses (Bovine Rotavirus A, Bovine Coronavirus, Bovine Torovirus and Bovine Pestivirus / BVDV), qPCR assays for the bacteria (Salmonella and Escherichia coli K99/F5) and protozoal parasites (Cryptosporidium and Giardia), which have been identified as the major pathogens involved in NCD in Australia¹. Faecal samples submitted to EMAI for routine diagnosis have been utilized to compare assays. Duplex assays are currently being optimized and validated.

Results and Discussion
Our results also show that faecal inhibitors may have a significant effect on the qPCR reaction and we are exploring options for dealing with this major limitation.

The next stage will involve multiplexing groups of target pathogens in real time PCR reactions (up to 4 agents) and LAMP. Internal controls will be used to monitor inhibition. Then, molecular techniques will be utilised to test environmental samples, to attempt to determine the pathogen load and or source / s of infection on beef properties.

Acknowledgements
Meat and Livestock Australia, Sydney
Staff at Elizabeth Macarthur Agricultural Institute (Virology, Bacteriology and Parasitology Laboratories) and the Shute Building, the University of Sydney, Camden

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INCREASING THE VOLUME OF TRANSFER MEDIA DOES NOT AFFECT PREGNANCY OUTCOMES OF IN-VIVO PORCINE EMBRYO TRANSFER

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First Year Masters Research (part time)

The results of previous porcine embryo transfer studies suggested that depositing the embryos in small volumes of culture medium (<1.6 ml) increased the efficiency of embryo transfer compared with large volumes¹. However, culturing embryos in a transfer syringe containing a small volume of medium (0.6 ml) significantly reduced their viability within 3 hours (T. Harris and C. Grupen, personal communication). We hypothesized that increasing the volume of Hepes-buffered Porcine Zygote Medium Version 3 (HPZM-3) from 1.4ml to 5ml during embryo transfer will have no effects on pregnancy outcomes and piglets born alive. Ovulation was induced in eighty-one donor sows synchronised at weaning using a regimen of equine chorionic gonadotropin and human chorionic gonadotropin. Sows were artificially inseminated on the day of first standing heat. Embryos were surgically recovered three to four days later. A total of 76 parity one and parity two recipients were synchronised at the same time as the donor animals. A mean (± SEM) of 24.1 ± 0.95 embryos was transferred to each recipient. Embryos were deposited in control sows (n=65) using a total of 1.4 ml HPZM-3 and in treatment sows (n=11) using a total of 5 ml HPZM-3. Increasing the volume of medium used to deposit the embryos from 1.4 ml to 5 ml did not affect the number of piglets born alive, still born or mummified. Farrowing rates also did not differ for the two groups (control 62%; treatment 64%; P>0.05). The ability to implant embryos in 5ml of media has the potential to greatly simplify the embryo transfer procedure as it eliminates the need for an embryologist at the implanting site whilst maintaining embryo viability during transport for longer durations.

References
MANAGING RISK: STUDIES OF THE BIOLOGY AND EPIDEMIOLOGY OF BLUETONGUE VIRUSES AND THEIR VECTORS IN NEW SOUTH WALES.

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Introduction
Bluetongue virus (family Reoviridae, genus Orbivirus1) is a vector borne arbovirus that can infect both domestic and wild ruminants. Bluetongue viruses (BTVs) are transmitted among ruminant hosts by Culicoides spp (Diptera: Ceratopogonidae) insect vectors. In recent years BTVs have developed a high profile because of unprecedented disease outbreaks in Western Europe, Scandinavian countries and England2,3. There are dramatic epidemiological and entomological differences between these outbreaks and the situation in Australia. Bluetongue disease is rarely observed in Australian livestock despite the distribution of BTVs throughout Northern and South Eastern Australia4. However, in the future, conditions that facilitate the convergence of the virus, the vector and the host in space and time may challenge our understanding of bluetongue disease ecology. The aims of this project are to: 1. conduct vector competence studies, 2. develop and evaluate rapid diagnostic tests for detection and quantification of insect vectors and BTV, 3. complete field studies to implement these diagnostic tools, and 4. conduct epidemiological studies on the range of BTV serotypes and insect vectors present in New South Wales.

Materials and methods
All molecular work is being conducted at the Elizabeth Macarthur Agricultural Institute, Menangle. Highlights include the use of automated DNA extraction and Real Time PCR. A unique feature of the DNA extraction protocol is non-destructive enzymatic digestion of insects, retaining original ‘voucher’ specimens for morphological referral. All entomological work is being conducted at the Tammworth Agricultural Institute and at Duck Creek Research Station, Lismore, NSW. Unique features of this work include devising methods to feed midges using a Hemotek blood feeding machine.

Results and discussion
The real time PCR assays for C. brevitarsis, C. wadai, C. fulvus and C. actoni have been evaluated for sensitivity and specificity. As proof of concept, the C. brevitarsis assay can readily detect one C. brevitarsis in a pool of mixed insects (n~110). Based on these results it should possible to detect a single C. brevitarsis in a pool of 10,000 other insects. The molecular assays will be evaluated on insect trap collections from the 2011−2012 season. At present, a real time PCR is being designed to distinguish the host species origin of midge blood meals. This molecular tool will be used to investigate the vector-host interaction in order to develop a greater understanding of bluetongue virus ecology.

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VARIATION IN SUPERVISORS’ EXPERIENCES OF VETERINARY PLACEMENTS IN THE YEAR 5 VETERINARY INTERNSHIP YEAR
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Introduction
Good teaching involves more than transmission of facts, concepts and principles. It should support deep approaches to learning and discourage surface approaches¹,². In the final year of The University of Sydney Bachelor of Veterinary Science degree students are engaged in an intern programme designed to encourage deep approaches to learning and student centred approaches to teaching but the extent to which placement supervisors adopt this approach is unknown. Phenomenographic studies have been widely used in past research on student learning and teaching¹,²,³ and this approach has been used to explore the variation in supervisors’ experiences of veterinary intern placements. This presentation reports on the results of a survey that can be considered to be a pilot study for this project.

Material and Methods
A survey administered to placement supervisors in 2007 explored supervisors’ conceptions of what it is that supervisors intend for students to learn and get out of supervision and how they approach their supervision. 39 responses were received. A phenomenographic approach was used to develop a robust set of categories for supervisors’ conceptions of learning outcomes and for approaches to supervision.

Results and Discussion
Preliminary results identified four distinct, empirically inclusive hierarchical categories of description for both conceptions of what students should learn and supervisors’ approaches to supervision. A qualitative difference was identified between categories B and C for both conceptions and approaches. Associations were revealed between conceptions and approaches suggesting that supervisors whose conceptions of learning outcomes are isolated and non-relational will be more likely to use supervisor centred, transmission approaches when supervising students. Those with conceptions that are cohesive and relational will be more likely to use approaches that are student centred and encourage autonomous engagement in veterinary practice. In the next stage of this project 118 completed surveys will be analysed using phenomenography to develop a robust and stable set of categories that describe the variation in supervisors’ conceptions of what supervision is about and how they approach their supervision. This will contribute to a body of knowledge that will assist in shaping curriculum design and teaching in the final intern year that reflects graduate attributes and contributes to developing graduates well prepared for transition for practice.

Acknowledgements
Dr John Baguley & Ms Melanie Robson for their assistance in contacting placement supervisors.

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THE GENETIC BASIS OF CANINE SEPARATION-RELATED DISTRESS DISORDER

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Introduction
Separation-related distress disorder is one of the most common behavioural disorders in our
domestic dogs, estimated at a prevalence of 14%[1] to 29% [2]. Affected dogs show behavioural
and physical signs of distress in the absence or perceived absence of a significant family
member. Common signs include vocalization, destruction, escape attempts, self trauma and
inappropriate elimination. Several risk factors have been recognised [3]. However, despite a long
held suspicion of a genetic basis, no study has identified the genes associated with the disorder.
Our aim is to map the genes underlying separation-related distress disorder in two breeds of
dogs – Labrador retrievers and golden retrievers.

Materials and Methods
Affected and control populations are identified using a validated behaviour questionnaire,
CBARQ [4]. Puppy carers complete the questionnaire for a subpopulation of guide dogs.
DNA is obtained from suitable identified candidates via saliva (Oragene).
Our target of 100 cases and 100 controls of each breed can then undergo whole genome
association analysis.

Results and Discussion
At present over 260 questionnaires have been completed, 65 of these through Guide Dogs
Victoria. Cases have been identified in 14% of pet/show dog population but only 1.5% of guide
dogs.
Further cases are required before genome-wide association studies can be performed. In the
meantime, a preliminary genetic association study of three candidate genes is commencing.
Statistical analysis of completed questionnaires may also provide further information about
interactions between the disorder, other behavioural problems and environmental factors. Initial
analysis suggests that dogs with a high score for separation-related problems also have a
higher score for attention-seeking behaviour and excitability compared to those dogs with a low
score for separation-related problems.

Acknowledgements
Goldia and Susie Lesue scholarship (2010), Neil and Allie Lesue scholarship (2011), Jean
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GENETICS OF DURABILITY IN RACING THOROUGHBREDS

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Introduction

Commonly referred to as wastage, high attrition rates in thoroughbred racing have been shown by a number of studies¹,². Attrition, often associated with injuries and diseases that occur during training and racing, has also been shown to occur prior to the start of the horse’s racing career¹,²,³. As animal welfare organizations continue to show concern for thoroughbreds in the racing industry, it is important that the racing industry further pursue ways of bettering the welfare of their equine athletes. The objective of this study is to assist this aim by providing a new tool for the racing industry through the development of a durability index: a selection index consisting only of traits associated with durability.

Materials and Methods

Performance data for all horses entered in a race or official barrier trial from the 1st Aug 2000 until 22nd Feb 2011 were provided by Racing Information Services Australia. Each record corresponded to a specific race or trial and included, among other things: horse, foal date, sex, sire, dam, trainer, owner, jockey, track, race/trial date, distance, track condition, money earned, weight carried, finish position, and stewards comments. Traits in preliminary analyses included career length, lifetime starts, and spells per year. Career length was defined as the time between an individual’s first race or trial and last race or trial. A spell was defined as a time period between consecutive races and/or trials that was greater than 90 days. Statistical analyses were performed using the statistical package R⁴.

Preliminary Results and Discussion

The sample used for analysis included 2,844,842 individual records. The total number of horses in the sample was 163,331. Summary statistics were calculated for the above traits and analyses estimating the genetic parameters of these traits are in progress. The development of a durability index that only includes traits with a demonstrable genetic impact on racing durability will allow owners, breeders, and trainers to factor durability into both management and breeding decisions. This will lead to racehorses that are better suited for the physical challenges that accompany racing and potentially decrease the amount of wastage in the racing industry.

References

SERUM GALACTOMANNAN DETECTION – EVALUATION OF A NEW DIAGNOSTIC TEST FOR UPPER RESPIRATORY TRACT ASPERGILLOSION IN CATS

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Third year of Master of Veterinary Studies (Coursework)

Introduction: Feline upper respiratory tract aspergillosis is an emerging disease that can be difficult to treat. Diagnosis usually requires positive fungal culture and /or cytological or histological identification of fungal hyphae in affected tissues. These tests involve invasive procedures and false negative and false positive results are possible. Measurement of serum galactomannan, a polysaccharide component of fungal cell walls, is a non-invasive, alternative test used in the early diagnosis of human invasive aspergillosis.

Aims: To evaluate the use of serum galactomannan measurement in the diagnosis of feline upper respiratory tract aspergillosis.

Method: Batched serum samples were tested for serum galactomannan using a one-stage immunoenzymatic sandwich microplate ELISA (Platelia™ Aspergillosis) in four groups of cats:

1. Cats with confirmed upper respiratory tract aspergillosis (n=13)
2. Cats with other upper respiratory tract diseases (n=15)
3. Cats without non-respiratory tract diseases treated with β-lactam antibiotics (n=14)
4. Healthy cats (n=45) – Juvenile cats (n=31) and adult cats (n=13)

Results: There was no significant difference in serum galactomannan measurements between the groups. High numbers of false positive results were identified in juvenile cats and in cats treated with β-lactam antibiotics. All affected cats with high serum galactomannan measurements were infected with a novel Aspergillus species.

Conclusion: Serum galactomannan measurement has a poor sensitivity and moderate sensitivity for the diagnosis of feline upper respiratory tract aspergillosis. The specificity of the test can be improved when known causes of false positive results are removed from the analysis.
INTERACTIONS OF E COLI O157 AND BOVINE INTESTINAL EPITHELium

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PhD, first year, full time

Introduction
Escherichia coli O157 causes severe and life-threatening disease in humans and has a low infectious dose. It can cause haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS), a leading cause of acute renal failure in children under five. Cattle have been identified as a reservoir of O157 and many outbreaks have been linked to the consumption of undercooked beef patties. O157 does not cause clinical disease in adult cattle. Cattle shed O157 in their faeces for varying durations and at varying concentrations¹. An obvious strategy for the reduction of O157 contamination in the food chain is the reduction of this shedding in cattle. The interaction of O157 with the epithelia of the bovine gastrointestinal tract (GIT) is not well understood. One of the first major questions to be raised regarding O157 in the bovine GIT was the location of any bacterial colonization and multiplication. This question has not yet been answered satisfactorily. Recent research has suggested that O157 forms attaching and effacing (AE) lesions in the GIT² and the rectoanal junction (RAJ) is the site of colonisation³. A better understanding of any potential tissue tropism of O157 and the interactions at the epithelium will aid the development of methods to reduce O157 shedding.

Aims and Objectives
There is a need for validation of the methods to quantify adherence and AE lesion formation in cell culture and in vitro organ culture (IVOC). These methods could then be applied to determine potential tropisms and host factors affecting colonization. The sampling of RAJ cells for use in adherence assays should provide a method for assessing the potential adherence of O157 strains in different animals and further investigating the tropism of different strains of pathogenic E. coli. The ultimate goal is to develop a simple test using RAJ swabbing to indicate the potential susceptibility of the animal to O157 colonization.

Acknowledgements
This work is funded by MLA project A.MFS.0247.

References
APPLICATION OF NEXT GENERATION SEQUENCING TO INCREASE EQUINE TRAIT MAPPING EFFICIENCY

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First year PhD research, full time.

The recent availability of a dedicated genotyping array has facilitated trait mapping in the equine. However, previous power calculations have shown that the density of genotypes remains sub-optimal for efficient mapping in ancient breeds, breeds with low linkage disequilibrium, large effective population size, or multi-breed sample sets\textsuperscript{1}. The projected number of markers required to perform efficient trait mapping in such projects is 320,000\textsuperscript{1}, greater than four-fold more than is provided by the current genotyping platforms. In order to increase the efficiency of gene mapping in the equine, an alternative genotyping approach is required which increases the number of genotypes returned per sample in a cost-effective manner.

Whole genome next generation sequencing offers one such alternative. Preliminary calculations suggest that an average four-fold genomic sequence coverage will be sufficient to genotype samples to the desired marker density. When combined with genotype imputation to increase the coincidence of typed markers between samples, powerful genome wide association analysis can be applied to identify candidate regions. The aligned sequence used to generate genotypes can further be applied to conduct fine mapping and mutation detection.

Deep sequence data of a Quarter Horse mare, generously donated by Texas A&M University, will be used in simulations to test the adequacy of four-fold sequence coverage for dense genotyping. A database of unique equine 25 base-pair sequence tags has been developed to increase genotype confidence by decreasing alignment ambiguity of paired-end reads. Genotype imputation will be applied following the generation of a representative equine haplotype panel by the recently formed Horse Genome Haplotype Committee. A straight-forward method to call, score and export sequenced and imputed genotypes will be developed. This approach will be applied in practice to a number of equine and canine genetic disease projects which are currently underway in our laboratory.

Acknowledgements

Scott Dindot, Texas A&M University College of Veterinary Medicine and Biomedical Sciences, College Station, Texas.

References

A DELICATE BALANCE: CHARTING THE RIGHT COURSE FOR A PEDIGREE DOG BREED.

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Abstract: Pedigree dog breeds are closed populations under selection by breeders who seek to "improve" many different traits including morphological traits (both aesthetic and functional) and behavioural traits (adaptive or maladaptive), and to reduce genetic disease. Selection works by locating and breeding disproportionately from candidate dogs whose alleles match such breeding objectives best. While pedigree dog stud books remain closed, selection can only utilise the genetic diversity which is present within the population, conserved from previous generations or arising anew from mutation.

Increasingly stringent selection reduces the proportion of breeding candidates judged suitable to produce progeny and can lead to a progressive loss of genetic diversity over time. Loss of that portion of genetic diversity which gives rise to genuinely undesirable traits through strong selection may be desired but it sometimes carries with it loss of benevolent genetic diversity; alleles which are neutral or even beneficial for the breeding objectives reducing in frequency or becoming lost. When benevolent allele diversity is lost faster than mutation can replace it, genetic heterogeneity, a characteristic associated with population health, is reduced. Selecting a breeding animal which carries no adverse alleles is mathematically unlikely, so stringent selection also perpetuates the adverse alleles of a few animals widely, increasing the likelihood of recessive disease in future generations¹.

As the intensity of selection must be balanced against these disadvantages, selection strength should be considered a limited, and only partly renewable, resource. If pedigree dog breed welfare is to be optimised, selection resources must be applied carefully to well chosen, evidence-based breeding objectives² and using accurate and evidenced-based candidate evaluation³.

References
TANGIBLE AND INTANGIBLE ECONOMETRICS OF EMERGENCY ANIMAL DISEASES

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Third Year, Full time

Introduction
Globalisation, technical advancement and increasing incidence of transboundary diseases have made animal health economics a priority for governments around the world¹. Protecting trade, preventing disease incursions (including zoonotic diseases), conditioning improvements in animal welfare standards and maintaining domestic food supply are paramount. Many economic appraisal methods exist for these elements; however their suitability and practicality are not consistent.

Materials and Methods
A review of currently used economic methodology is undertaken to ascertain the limitations and benefits of these assessment processes. Research into economic assessment tools used in areas other than animal health is also being undertaken, to determine their suitability for application at the national level as decision support tools for policy making. Development of a framework that can be applied as an economic assessment tool for emergency animal diseases within Australia will occur and focus groups will be selected to review the framework to determine potential aptitude in comparison with currently used methods.

Results and Discussion
Of the nine economic assessment tools currently reviewed, none are without limitations. Cost-Benefit Analysis (CBA) is the most widely used in research and practice, as it can be a flexible and integrative tool. However the CBA of conducting any economic assessment within a sector of animal health can prove inhibitive, with data requirements often a limiting feature. Newer methods of economic assessment borrowed from areas such as ecological and environmental economics include assessment methodology for intangibles and appear to work more consistently under conditions of uncertainty.

Acknowledgements
Michael Ward (PhD, FACVS), Chair, Veterinary Public Health & Food Safety, Faculty of Veterinary Science, The University of Sydney.
Graeme Garner, Manager, Epidemiology Program, Office of the Chief Veterinary Officer, Department of Agriculture, Fisheries and Forestry, Canberra.

References
USING SEQMONK TO COMPARE GENE EXPRESSION BETWEEN PERIPHERAL NERVE AND DEVIL FACIAL TUMOUR DISEASE TISSUES

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Introduction
The Tasmanian devil is facing extinction because of a contagious cancer – Devil Facial Tumour Disease (DFTD)¹. The clonal cell line originated from a Schwann cell or Schwann cell precursor². Therefore, we have generated transcriptomic data to compare gene expression between peripheral nerve cells and DFTD cells. I have used the software package SeqMonk to identify genes that are differentially expressed between the two samples with the aim of identifying genes involved in DFTD.

Materials and Methods
Around 20 to 30 millions of illumina reads of each sample were aligned to the recent version (7.1) of devil genome and then imported into SeqMonk. The genome annotation (version 2) I used was received from Dr Papenfuss’s group in Melbourne. Genes appeared significantly over-expressed (at least 4 fold) were selected for further analysis, including gene ontology functional clustering and pathway analysis.

Results and Discussion
2007 genes contained at least one exon that was expressed at a significantly higher level in tumour samples than in peripheral nerves. 152 of these genes are known to be involved in the cell cycle, and therefore were selected for further interrogation. Furthermore, expressed regions in introns were found constantly and the significance of these transcripts will be discussed.

Acknowledgements
Emily Wong, Postdoc Fellow, Faculty of Veterinary Science, The University of Sydney, Sydney.
Katherine Belov, Associate Professor, Faculty of Veterinary Science, The University of Sydney, Sydney.
Anthony T Papenfuss, PhD, Walter+Eliza Hall Institute of Medical Research, Melbourne.

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