PG Research Day

BOOK OF ABSTRACTS

RESEARCHER ASSOCIATION

Royal Veterinary College
University of London

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FOREWORD

Welcome to Postgraduate Research Day 2016.

This event is now in its third year and is being held for the first time at the Hawkshead campus. The aim of the day if to provide our PhD students with a collegiate forum to present their work, and for you to learn more about the diverse range of postgraduate research that is being conducted at the College. There will be two parallel streams of talks by final year students throughout the morning and an additional one in the afternoon. There are prizes to be won for the best presentations as voted for by the audience, so do please come along to as many talks as you can and complete the scoring sheets provided in the rooms! First year students will be presenting their projects in poster format and there will be a dedicated poster session after the lunch break during which the poster presenters would be delighted to chat to you about their work. Poster prizes will be awarded after thorough evaluation of all entries by a panel of six judges.

I must say a big 'Thank you' to all those who have contributed to organising and running this event. Particular thanks go to Mazdak Salavati, Rowena Packer and Marta Farré Belmonte of the Researchers Association; Maxine Esser, Lisa Matamala-Shaw and Shivanthi Manickasingham of the Graduate School, Patricia Latter as one of Impact Statement judges and the poster judges. Of course the Postgraduate Research Day would not be successful without an audience, so thank you for taking part and providing our PhD students with the opportunity to share their work with you.

I wish you all an enjoyable and productive day. If you have any feedback or suggestions for the event, please feel free to contact any member of the Graduate School team - we'd be pleased to hear from you.

Kristien Verheyen
Head of Graduate School
May 2016

SCHEDULE FOR THE DAY

Time	Conference suite room 1-2		Conference suite room 3-4	
	Title	Speaker	Title	Speaker
10:00 - 10:05	**Introduction**	**RA**	**Introduction**	**RA**
10:05 - 10:20	The application of marketing methodology to the advocacy of on-farm biosecurity: Targeted strategies	HN. Pao	Transcriptional regulation of the equine chorionic gonadotrophin beta subunit	J. Read
10:20 - 10:35	Does endothelial cell dysfunction underlie the predisposition to pasture associated equine laminitis	EJT. Finding	Gait dynamics in spiders	MA. Reeve
10:35 - 10:50	What is the recipe for diabetic remission in the cat	RF. Gostelow	Biomechanical modelling / motion mapping of computer generated quadrupeds	K. Abson
10:50 - 11:05	Objective Assessment of Thoracolumbar Movement and body lean angle in Subjectively Sound Horses and horses with Hindlimb Lameness	L. Greve	Walking with giraffes – ground reaction forces and kinematics	CK. Basu
11:05 - 11:20	Metabolic profiling in canine epilepsy	TH. Law	Developing, testing and optimising a simulation of mouse hindlimb locomotion	J. Charles
11:20 - 11:45	**Coffee break**			
11:45 - 12:00	Assessing the welfare of horses in the UK'	L. Crowther	PPARβ/δ-mediated regulation of cellular metabolism: implications for endothelial cell dynamics.	A. Faulkner
12:00 - 12:15	The effects of Enterococcus faecium NCIMB 10415/SF 68 in the gastrointestinal tracts of healthy dogs	LA. Matthewman	The effect of temperature rise upon immunity and susceptibility to infection in fish	S. Bearder
12:15- 12:30	Evaluating the anti-diabetic potential of the herb Hymenocardia acida	I. Ezeigbo	Development of a novel approach to solve genome assemblies' jigsaw	J. Damas

			puzzles	
12:30 - 12:45	Estimation and monitoring of the frequency of selected endemic diseases and conditions of dairy cows in Great Britain	M. Velasova	The epidemiology of bovine tuberculosis at a wildlife-livestock interface in Uganda	N. Meunier
12:45 - 13:00	Multidisciplinary approach for improving diagnosis and treatment of canine neuropathic pain.	S. Sanchis Mora	The isolation and identification of potential vaccine antigens against Dermanyssus gallinae, the poultry red mite	J. Pritchard
13:00	Lunch break			
13:30 - 15:00	Poster viewing (should be installed from 10:00 am)			
15:00 - 15:15	Impact of foot and mouth disease on smallholders of the Andean Region	G. Limon Vega	Identification and analysis of live attenuated vaccine candidates of Mycoplasma hyopneumoniae	AS. Deeney
15:15 - 15:30	Integrated use of epidemiological tools to understand African swine fever transmission	C. Guinat	Development of novel biotherapeutics for S. aureus infection'	SE. Woodman
15:30 - 15:45	Translating Theory into Practice: Targeted tuberculosis control in meerkat of the Kalahari	ST. Patterson	Potentiation of Polyhexamethylene biguanide and nadifloxacin activities against intracellular MRSA by nanoparticles formulation	NF. Kamaruzzaman
15:45 - 16:00	Use of Functional Electrical Stimulation to re-animate the larynx in Recurrent Laryngeal Neuropathy	J. Perkins	Identification and characterisation of myeloid cell populations in porcine tonsils	F. Soldevila Casals
16:00 - 16:15	Skeletal effect and signalling mechanism of Glucagon-like peptide 1 agonist in bone	M. Pereira	Immunosenescence in geriatric dogs and impact on vaccination	A. Holder
16:15	Cheese and wine reception			
16:45	Prize giving and closing			

ORAL PRESENTATION ABSTRACTS

Understanding anatomical movement through animations

Karl Abson

Human observation is relied upon in decision making processes. Issues however with observation of movement and its subsequent understanding have been identified. In horse lameness for example, multiple influencing factors currently inhibit decision making; observer experience (Keegan et al, 2008, Keegan et al., 2010), observer bias (Arkell et al, 2006) and the limitations of the human eye (Parkes et al, 2009). Furthermore this issue is not confined to one sector, in the creation of digital motion the uncanny valley (Burleigh et al, 2013) and the "seduction of realism" (Hodkinson. 2009) have proven problematic as movement must be understood by those re-creating it.

Technology such as motion capture can prove useful in overcoming this challenge in high priority areas but not in typical everyday situations (Skrba et al, 2009). It could however offer the ability to create animations and training material which can then inform human based methods which are suitable (Abson and Palmer, 2014).

This study aims to provide an investigation into the possibilities and capabilities of complex biomechanical animations. It provides an investigation into what can be seen and how well it can be seen by varying variables such as camera angle, film speed and bone movement. This information on what makes a good animation is coupled with an analysis of students' views around the use of animations and practical analysis of animation creation methods.

This knowledge provides answers to many questions necessary for the successful creation of digital motion. The study provides useful insights for training material and course design, in understanding expectations and the suitability of this facilitation method. The main impact of this work is a contribution towards the training of practitioners and subsequent improvement of their craft. Further impacts include the visualisation of research results and subsequent greater public accessibility.

Walking with giraffes – ground reaction forces and kinematics

C. Basu

Giraffes (*Giraffa camelopardalis*) are ruminant artiodactyls whose large body mass combined with long, gracile limb and neck proportions render them highly derived in comparison to sister taxa. Neck and limb elongation is predicted to have functional consequences with respect to locomotion in giraffes. Previous studies have been confined to describing kinematics from a small number of trials, usually with single individuals. We have measured ground reaction forces and simultaneous 2D kinematics from three adult giraffes, from over 100 walking trials. Data were gathered over several days in an outdoor zoo enclosure. Giraffes were encouraged to walk over a forceplate array at their preferred speeds. We describe basic kinematic parameters (including stride length, stride frequency, duty factor and preferred speed), and relate these to peak vertical and horizontal forces. The three adults of similar size demonstrated a singlefoot in lateral sequence gait, using a preferred speed of 1.2m/s. At this velocity, the mean (of forelimb and hindlimb) duty factor was 0.67, peak forelimb vertical ground reaction force was 0.76x body weight, and peak hindlimb vertical force 0.4x body weight. Mean stride length was 2.3m, and mean stride frequency 0.5 Hz. Changes in walking speed were achieved both with an increase in stride length, and to a lesser extent an increase in stride frequency. The long neck of giraffes surely provides a high moment of inertia, which may in turn influence the acceleration and deceleration forces of the forelimb. We hypothesize that oscillations in neck angle are linked to the forelimbs' horizontal forces, and find that the peak neck flexion coincides with peak braking force in early stance, but is disconnected from peak propulsive force.

* This abstract was accepted although it was submitted after the deadline

The effect of temperature rise upon immunity and susceptibility to infection in fish

Siobhan Bearder[1], Imelda M McGonnell[1], Brendan W Wren[2] & Rachel A Lawrence[1]

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Environmental temperature changes are likely to alter the susceptibility of wild and farmed fish to infectious disease. Recent studies have shown that important fish species have an increased susceptibility to the aquatic pathogen, *Streptococcus iniae* at higher temperatures. We have established a model of this by exposing zebrafish larvae to *S. iniae* via immersion and microinjection at the normal zebrafish rearing temperature of 28°C and a higher temperature of 31°C. Initial experiments showed that immersion of zebrafish larvae in *S. iniae* had little impact on survival and did not represent the mortality observed in aquaculture, regardless of infection dose. Hindbrain microinjection of *S. iniae* resulted in a greater mortality in larvae reared at the higher temperature. *S. iniae* disseminated throughout the host. The locomotor activity of microinjected larvae was measured to further study the impact of *S. iniae* infection. Infected larvae at 31°C showed a significantly lower frequency of movement when compared to infected counterparts. Preliminary assessment of neutrophil responses suggested that larvae reared at 31°C have less neutrophils infiltrating the head than larvae reared at 28°C, whereas the concentration of neutrophils in the tail remained consistent. Zebrafish larvae reared at a higher environmental temperature and infected with *S. iniae,* show a decreased movement in a possible effort to conserve energy, and evidence of downregulation of the immune response and an increased mortality. Overall, a robust model using microinjection of *S. iniae* into zebrafish larvae has been developed for assessing the effect of environmental temperature on the susceptibility to infection.

*** This abstract was accepted although it was submitted after the deadline**

Creating, testing and optimising a simulation of mouse hindlimb locomotion

James P Charles[1,2], Ornella Cappellari[1], Andrew J Spence[3], Dominic J Wells[1], John R Hutchinson[2]

Terrestrial animals are able to move over various terrains in a stable and controlled way. This occurs through interactions between nerves, muscles and the environment, however their inherent complexity means they are not yet understood. Here we describe the creation of a biomechanical model of the hindlimb of the mouse, an animal commonly used in studies related to treatments for neuromuscular disorders and movement control. With this model, it will be possible to explore the intricacies of vertebrate locomotion in new detail, and as mice are thought to be close to the morphology of early mammals, it may give insights into the evolution of this lineage.

To develop the model, 39 muscles of the hindlimb were identified through I_2KI enhanced microCT scanning, which allowed muscle attachment points to be determined. These were then dissected to determine their architecture, which was used to estimate their force-generating and length-change potential. A sensitivity analysis supported its validity.

To simulate mouse locomotion, the hindlimb kinematics and ground reaction forces throughout a single representative stride were measured experimentally and added to the model. It was then possible to calculate individual muscle moments around each joint as well as predict patterns of muscle activation during running. Using a forward dynamics approach, we can predict the responses within the hindlimb to sensory or motor perturbations. These responses will be compared to experimental data, where optogenetics will be used to apply perturbations during movement. This systems approach may give valuable and novel insights into neuromuscular movement control, both within small rodents and potentially more generally within terrestrial vertebrates.

Importantly, all of this potential for biomechanical simulation and insight into neuromotor control within animals such as the mouse depends on the high-fidelity imaging of musculoskeletal anatomy enabled by I_2KI enhanced microCT scanning.

Assessing the welfare of horses in the UK

L Crowther[1,2], S Abeyesinghe[1] and C Burn[1]

[1] Animal Welfare Science and Ethics Group, Royal Veterinary College, UK
[2] The Horse Trust, Princes Risborough, UK

Welfare assessments are routinely performed by professionals and lay-people in a range of contexts. This presentation discusses the results of research into factors affecting the welfare of horses in the UK, and stages in the development of a standardised Welfare Assessment Protocol (WAP). Veterinary surgeons, welfare officers and welfare scientists with expertise in assessing equine welfare were invited to participate in an iterative survey that aimed to identify important animal-based welfare indicators suitable for inclusion in a WAP intended for field use. Thirty five animal-based measures were suggested, which included alertness, pain, resting behaviour, eye conditions, lameness, body condition, wounds, hoof condition, human-horse relationship, affiliative behaviours, and location of the horse in relation to conspecifics. A WAP was produced using information from published literature, field experience and the iterative survey. It was tested on 83 rescued and retired working horses resident at welfare charities. The aim was to determine the prevalence of each welfare indicator and identify associations between the indicators. Diarrhoea, lameness, wounds, limb swelling, hoof cracks, and a neutral or positive response to an approaching person were observed most frequently. Significant associations were identified between different body postures, which might be representative of different alert/responsive states. An online survey aimed to identify important human factors and welfare indictors that influence the decision to euthanase horses. The most common reasons for euthanasia were concerns over current/future welfare state, illness or disease, and veterinary opinion suggesting a poor prognosis. The most prevalent indicators that it was the right time to euthanase the horse were lameness, difficulty standing up/lying down or rolling, increased or ongoing performance of pain behaviours, and being unresponsive to things the horse would normally respond to.

Development of a novel approach to solve genome assemblies' jigsaw puzzles

Joana Damas[1], Rebecca E. O'Connor[2], Marta Farré[1], Henry Martell[2], Anjali Mandawala[2], Sunitha Joseph[2], Katie E. Fowler[3], Cai Li[4], Darren K. Griffin[2], Denis M. Larkin[1]

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[4] China National GeneBank, BGI-Shenzhen, Shenzhen, China

With genomes assembled to chromosome-level we can confidently study, for instance, the role of chromosomal evolution in speciation or the effects of long-range *cis* and *trans* interactions in gene regulation. However, for most genomes sequenced using next-generation sequencing (NGS) technologies we do not have one contiguous sequence per chromosome. Instead, each genome assembly consists of thousands of small DNA fragments called 'scaffolds'. To assign and order the scaffolds of a *de novo* assembled genome to its chromosomes, we developed a new genome mapping and scaffolding methodology. Our approach consists of four steps: (1) construction of predicted chromosome fragments (PCFs) using the reference-assisted chromosome assembly (RACA) algorithm; (2) verification by polymerase chain reaction (PCR) of a subset of scaffolds that are critical for revealing species specific chromosome structure; (3) creation of a refined set of PCFs using RACA with adapted parameters based on the PCR results; and, (4) placement of the refined PCFs on the species chromosomes using fluorescence *in situ* hybridization (FISH) of bacterial artificial chromosome (BAC) clones containing evolutionary conserved genome intervals. We applied our new methodology, up to step 3, to 18 avian scaffold-level assemblies. We were able to reduce the number of fragments of these genome assemblies up to 94%, significantly increasing the continuity of the genome. For peregrine falcon and rock pigeon, completed to step 4, we were also able to detect intra- and inter-chromosomal rearrangements not previously identified. Our approach proved to be a fast and efficient method to upgrade fragmented scaffold-level assemblies to chromosomal level. The improved avian genome assemblies will provide a foundation for a detailed study of chromosomal evolution in avian and non-avian reptiles, as well as the role of chromosomal rearrangements in the emergence of new phenotypes in evolution.

Investigating naïve interactions between alveolar macrophages and *Mycoplasma hyopneumoniae*

Alannah Deeney

The functional aspects of interactions between *Mycoplasma hyopneumoniae* and alveolar macrophages have not been well characterised. This work aimed to establish how primary porcine alveolar macrophages (PAMs) respond to *M. hyopneumoniae* in a naïve *in vitro* setting. To establish if PAMs could phagocytose *M. hyopneumoniae*, a gentamicin protection assay and flow cytometry phagocytosis assay were set up. After gentamicin treatment in the protection assay, viable control bacteria (*E. coli* DH5α) were recovered from inside PAMs, indicating PAMs were able to phagocytose. However, no viable *M. hyopneumoniae* CFU were recovered from inside PAMs after external bacteria had been killed. In the FACS phagocytosis assay PAMs treated with or without cytochalaisin D (CCD) were incubated with *M. hyopneumoniae* transformed with green fluorescent protein (GFP). CCD is a fungal toxin that inhibits actin polymerisation, thus bacteria can bind to the PAM surface but actin-dependent phagocytosis is inhibited. If PAMs phagocytose GFP bacteria the fluorescence intensity of untreated PAMs will be higher than CCD treated PAMs. Untreated PAMs incubated with GFP *M. hyopneumoniae* did not have higher GFP fluorescence intensity than CCD treated PAMs. The tentative conclusion from this work is that *M. hyopneumoniae* makes a physical association with PAMs but is not phagocytosed.

Extracts of *Hymenocardia acida* Ameliorate Insulin Resistance in L6 myotubes

Ihechiluru I. Ezeigbo

Insulin resistance (IR) in skeletal muscle is a primary defect underpinning the development of type 2 diabetes (T2D). Muscle IR is typically associated with obesity and causatively linked with increased circulating levels of saturated fatty acids (FAs). Few licenced products improve insulin sensitivity and these are not available cheaply in the developing world. In parts of West Africa, decoctions, including those of *Hymenocardia acida*, are widely employed for the treatment of diabetes; however, there is limited scientific evidence to support their use. Thus, I assessed the potential for *H. acida* extracts to ameliorate FA-induced IR in L6 myotubes.

H. acida extracts were prepared in methanol (MeOH) and chloroform ($CHCl_3$). The methanol extracts were further subjected to solid-phase extraction (SPE) on C18 silica using ratios of $MeOH:dH_2O$ from 0 to 100%, yielding 15 sub-fractions. Sub-fractions were dried and analysed using ^1H-NMR spectroscopy. The effect of crude extracts or SPE sub-fractions on the viability of myotubes was assessed using resazurin. Subsequently, myotubes were treated with palmitic acid (PA) to generate IR and incubated for 16 h with a range of concentrations of extracts or sub-fractions that did not impair viability. The effects on insulin sensitivity were assessed using uptake of 2-deoxy-$[^3H]$-glucose ± 100nM insulin.

Treatment with PA abolished insulin-stimulated glucose uptake in L6 myotubes, as expected. Co-incubation with pioglitazone (50 µM) or the MeOH extract (200 µg/ml) significantly restored insulin-stimulated glucose disposal (by 66.7%; p=0.022 and 81.9%; p=0.004 respectively), while the $CHCl_3$ extract and SPE sub-fractions were not effective.

Thus, MeOH extracts of *H. acida* ameliorate IR in skeletal muscle cells *in vitro* and thus merit further investigation as potential anti-diabetic substances.

* This abstract was accepted although it was submitted after the deadline

Regulation of endothelial cell metabolism by PPARβ/δ and its impact on angiogenic function.

<u>A. Faulkner</u>

It has long been recognised that cells undergoing rapid and dynamic alterations in growth and proliferation, such as occurs during angiogenesis - the growth of new vessels from pre-existing vasculature, exhibit significant alterations in their metabolism. A central feature for most cell types is a significant up-regulation in aerobic glycolysis (Warburg-like metabolism). It is also becoming clear that the signalling pathways regulating such alterations are themselves receptive to reciprocal feedback exerted by metabolites through post-translational modifications such as acetylation and phosphorylation. The importance of these metabolic communications in ECs to facilitate their angiogenic function is only recently becoming clear. Its position as a metabolic hub and its known interaction with other metabolic regulators, including sirtuin's, may allow the PPARβ/δ nuclear receptor to act as a link between EC metabolism and angiogenesis-associated signalling to facilitate the dynamic angiogenic phenotype. The aim of this thesis was to characterise the metabolic response of HUVEC to PPARβ/δ activation and identify potential pathways facilitating PPARβ/δ-mediated stimulation of EC dynamic activity.

Pharmacological activation of PPARβ/δ with GW0742 did not stimulate HUVEC migration but significantly increased the number of capillary-like tubes formed in the *in vitro* tube formation assay. At the mRNA level, GW0742-induced capillary-like formation led to a significant shift in LDH isoform expression favouring lactate production whilst supressing genes involved in mitochondrial fatty acid β-oxidation. These changes were associated with an elevated glycolytic flux with no change in glucose oxidation. Consequently, pharmacological inhibition of key glycolytic enzymes (PFKFB3 and LDH) significantly reduced HUVEC ability to form capillary-like structures. Modulation of cell function by PPARβ/δ has been shown to have a degree of SIRT1-dependency. Pharmacological inhibition of the NAD^+/SIRT1 axis significantly reduced the ability of HUVEC to form capillary-like structures in response to GW0742 stimulation. Whilst reducing NAD^+ bioavailability by inhibition of NAMPT (rate-governing enzyme in the NAD^+ biosalvage pathway) showed a partial inhibition of GW0742-mediated capillary-like formation, the direct inhibition of SIRT1 caused complete abolition of GW0742-induced capillary-like structures. The acetylation status of FOXO1, a common SIRT1 target and regulator of angiogenesis, remained unchanged in GW0742-treated HUVEC, suggesting that FOXO1 is not a relevant target for SIRT1 in this setting. Although no evidence of mitochondrial biogenesis could be detected, suggesting that the SIRT1/PGC1a pathway was not activated, there was significant up-regulation in MFN2, suggesting possible changes in mitochondrial morphology or interaction with other organelles that may or may not have an important role to play in PPARβ/δ-mediated angiogenesis.

Together these studies suggest that PPARβ/δ-mediated angiogenesis is a SIRT1-dependent process that facilitates an increase in glycolysis whilst suppressing fatty acid β-oxidation. Further studies are needed to ascertain the exact nature of the SIRT1-dependency observed in these studies.

Does endothelial dysfunction underlie the predisposition to pasture associated equine laminitis?

EJT Finding[1], J Elliott[1], PA Harris[2], NJ Menzies-Gow[1]

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Laminitis is a painful condition of the hoof affecting up to one third of the equine population. Some animals are predisposed to recurrent episodes of laminitis; the characteristics of this population are similar to those of people predisposed to cardiovascular disease. Endothelial dysfunction is a feature of the predisposition to human cardiovascular disease and may also play a role in the pathogenesis of laminitis. Microvesicles are both markers of and cause endothelial dysfunction. Investigating endothelial function in the horse will help clarify potential risk factors for laminitis, leading to development of better tests to identify predisposed animals, allowing targeted introduction of preventative management.

In vivo methods used in humans to measure endothelial function were investigated but could not be translated to the horse. Circulating biomarkers of endothelial function were measured and showed variation with season only. Inhibition of nitric oxide (NO) synthesis resulted in over 2 fold greater reduction in NO in ponies with than without predisposition to laminitis. Techniques to measure equine microvesicles were developed and the effect of microvesicles on equine endothelial function in vitro was studied using endothelial cell culture and microvascular wire myography. Microvesicles from laminitis predisposed ponies increased production of vasoactive mediators by endothelial cells. Microvesicles from both groups of ponies reduced endothelium dependent vasodilatory responses of laminar vessels.

Endothelial function differs with predisposition to laminitis and markers of endothelial function vary with season. Laminitis occurs predominantly in spring and summer; therefore disease predisposition is likely to be a combination of seasonal environmental factors and individual animal factors such as endothelial function. The effect of microvesicles on endothelial function should be studied in different seasons to investigate this relationship further. The molecular mechanism of this effect should be established with further investigation of the NO pathway, potentially by studying the relationship between NO and vasoconstrictors.

What is the Recipe for Diabetic Remission in the Cat?

Ruth Gostelow

Diabetes mellitus (DM) is increasingly common in cats, with most cases resembling human Type 2 DM. Treatment of feline DM is challenging, but some cats enter remission and stop antihyperglycaemic medication. This project aimed to identify factors associated with feline diabetic remission, as this knowledge would be highly beneficial for veterinarians, pets and owners.

Firstly, the existing level of evidence on influence of treatment type on remission rate was assessed using a Cochrane–style systematic review. Evidence was poor to moderate with common sources of bias including lack of randomisation and blinding among trials. A 1-year, randomised trial was then performed to compare remission rate using twice-daily (BID) protamine zinc insulin (PZI) or BID insulin glargine in recently-diagnosed (<5 months) diabetic cats. Cats were excluded if pre-enrolment screening identified glucocorticoid/progestogen administration, clinical pancreatitis, hyperthyroidism, patient non-compliance, hypersomatotropism, or conditions that affected ability to comply with trial commitments. Cats received a low-carbohydrate diet and overweight cats (body condition score >5/9) received a caloric intake for weight loss. Proportion of cats entering remission in each group was compared using Kaplan-Meier product limit estimates and log rank test, and predictors of remission were identified using Cox proportional hazards modelling (CPHM). Significance was set at $p<0.05$.

Forty-six cats were recruited (PZI = 22, Glargine = 24). Group were similar at baseline. Eight glargine-treated cats (33%) and five PZI-treated cats (23%) entered remission (p = 0.37). Univariate CPHM revealed increased likelihood of remission with body mass index ≥ 25% at enrolment (HR 3.3; 95% CI 1.1-10; p=0.04), and with ≥2% weight loss at the 1-month time point (HR 12.3; 95% CI 3.4–45.3; p=0.0002). Weight loss at 1-month was an independent predictor of remission in multivariable analysis (HR 12.9 95% CI 2.8-58.8; p=0.0009). No cat with BMI ≥25% achieved remission if gaining weight at 1-month.

Objective assessment of thoracolumbar movement and posture in subjectively sound horses and those with hindlimb lameness

Line Greve[1,2]*, Thilo Pfau[2] and Sue Dyson[1]

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Back pain affects many people and horses. Injuries in the limbs/legs negatively affect welfare and may predispose to back pain. Musculoskeletal pain impairs postural control and stability in man. Increased body lean has been observed during lungeing in lame horses. Half of the general sports horse population presumed to be sound by their owners exhibit gait patterns consistent with lameness. However, the mechanism linking lameness, back pain and body lean is not well understood, and accurate/repeatable tools for early detection of lameness are essential to develop prevention strategies.

With sensor-based gait assessment we can investigate factors, which are likely contributing to the interaction between limb pathology, thoracolumbosacral kinematics and impaired postural control/stability under 'real-life' conditions in horses with pathological and non-pathological gaits. We use an *'inverse model'* of how lameness influences thoracolumbosacral kinematics and leaning patterns by measuring the changes when lameness is abolished by diagnostic analgesia.

Non-pathological gait: Moving on a circle induces changes in thoracolumbosacral movement compared with moving in straight lines, associated with alterations in the hindlimb gait. Young horses exhibit poorer postural control/stability (i.e., lean more than expected based on speed and radius into the circle) when moving on a circle compared with older horses. The level of training and musculoskeletal strength determines how vertically orientated the equine body can be on a circle (e.g., horses which worked correctly when ridden leant less than expected based on speed and radius compared with horses that did not work correctly).

Pathological gait: Lameness causes thoracolumbosacral asymmetry and decreased range of motion (stiffness) that are both altered immediately by improvement in lameness using diagnostic analgesia. Some horses adapt to lameness by increasing body lean angle on a circle, which can be reduced immediately by improvement in lameness using diagnostic analgesia.

Clinical relevance: Thoracolumbosacral stiffness and altered leaning pattern on circles may be signs of lameness.

Integrated use of epidemiological tools to understand African swine fever transmission

Claire Guinat, Dirk Pfeiffer, Linda Dixon

Since its first detection in Georgia in 2007, African swine fever (ASF) continues to pose a major threat to pig farming in European countries. This PhD project aims to develop new knowledge about the most important drivers of ASF virus (ASFV) transmission dynamics and their interactions at different scales - from individual host to national level – using data from Caucasus, Eastern Europe and the Baltic countries.

Extensive literature review indicated that close contact between infectious and susceptible pigs was the main ASFV transmission routes. Experimental infections of domestic pigs with a highly virulent Georgia ASFV strains revealed high levels of virus titres in blood, secretions and excretions. All pigs became infected and died in less than two weeks, suggesting a limited potential of this strain to cause persistent infections. The transmission dynamics were quantified from these experimental trials using stochastic compartmental models, estimating the pig-to-pig R_0 at 2.8 within-pen and 1.4 between-pen. Similar models were fitted to pig mortality data collected during ASF outbreaks in the Russian Federation (RF), estimating the within-herd R_0 at 7.4 to 14.7. Social surveys investigating farmers' ASF knowledge highlighted areas of focus for future preparedness campaigns to increase the likelihood of early detection. Exploration of pig trade movements identified geographical regions, time periods and premises with increased opportunities for disease introduction and spread. Finally, this information was used to develop the structure and parameterise a between-farm transmission model which simulated the number of premises at risk of infection between disease incursion and detection.

Despite some potential biases that are discussed, this PhD project presents an integrated use of a diverse but complementary set of epidemiological tools to explore ASFV transmission and the results are suitable to inform policy development.

Immunosenescence in Geriatric Dogs and Impact on Vaccination

Angela Holder, Brian Catchpole and Donald Palmer

Immunosenescence is the gradual deterioration in immune system function associated with ageing. This decline is partly due to involution of the thymus, which leads to a reduction in the output of naive T cells into the circulating lymphocyte pool, thus causing an expansion of existing memory T cell populations to occur. This in turn can lead to reduced diversity in the T cell repertoire and impairment of immune responses to novel antigens, such as during infection and vaccination. In dogs the processes of immunosenescence are not well characterised. Therefore, the aims of this study were to examine age-related changes in thymic output and T cell repertoire in the dog, and to investigate the influence these changes might have on response to vaccine in geriatric dogs.

Thymic output was estimated using a quantitative PCR assay to measure signal joint T cell receptor excision circles (sj-TRECs), a molecular marker for recent thymic emigrants. There was an age-related decline in sj-TREC values in Labrador retriever dogs, which was most evident between the ages of 1 and 5 years. Examination of dog breeds with different life expectancies revealed the decline in sj-TREC values began at a younger age in large, short-lived breeds (Burnese mountain dogs, Great Danes and Dogue de Bordeaux) compared to small, long-lived breeds (Jack Russell terriers and Yorkshire terriers).

Diversity in the T cell repertoire was investigated by analysing the expression of T cell receptor (TCR) Vβ gene families in canine blood. Size spectratyping, by capillary electrophoresis, was used to look for evidence of clonal expansion/deletion. In Labrador retrievers, older dogs (10+ years) were found to have an increased number of TCR Vβ gene spectratypes demonstrating a skewed distribution, compared to young dogs (<3 years). This suggests that there is an age-associated disturbance in the diversity of the T-cell repertoire in Labrador retrievers.

Potentiation of Polyhexamethylene biguanide and nadifloxacin activities against intracellular MRSA by nanoparticles formulation

Nor Fadhilah Kamaruzzaman & Liam Good

Department of Pathology and Pathogen Biology, Royal Veterinary College

Introduction: The treatment for intracellular infections remains a challenge due to inadequate potency of antimicrobials inside host cells. The aim of this study is to improve overall potency of two topical antimicrobials, Polyhexamethylene biguanide (PHMB) and nadifloxacin against intracellular methicillin-resistant *Staphylococcus aureus* (MRSA) by formulation as nanoparticles. **Methodology**: PHMB/nadifloxacin nanoparticles were prepared by self-assembly method, followed by physical characterization using fluorescence spectrophotometer, dynamic light scattering, and transmission electron microscopy. Toxicity of nanoparticles towards keratinocytes was evaluated by measuring released of lactate dehydrogenase, followed by cell viability assessment using resazurin assay. The antimicrobial activities of nanoparticles and the free components, alone and in-combination were tested against intracellular MRSA in infected keratinocytes followed by measuring re-growth of the bacteria after 72 hours of treatments. Recovery of infected keratinocytes was measured by microscopy and cells counting. **Results and discussion**: Formulation of PHMB and nadifloxacin produced sphere nanoparticles with size 200 nm ± 50 nm with polydispersity index 0.3. The nanoparticles showed less toxicity than the free PHMB towards keratinocytes. At lower dosage, the nanoparticles showed more potent antimicrobial activity against intracellular MRSA and prevent re-growth of the bacteria, in comparison with individual antimicrobial tested alone or in combination (without pre-formulation as nanoparticles). Finally, the nanoparticles also improved recovery of infected keratinocytes. **Conclusion:** Formulations as nanoparticles were more beneficial than the free drug or in combinations, without pre-formulation as nanoparticles. **Keywords**: Nanoparticles, nanomedicine, PHMB, nadifloxacin, intracellular MRSA

Metabolic Profiling In Canine Epilepsy

Tsz Hong Law

Epilepsy is the most common chronic neurological disorder in both humans and dogs and despite appropriate antiepileptic drug (AED) treatment, approximately 30% of humans and dogs with epilepsy continue experiencing seizures. Furthermore, AED related side effects also contribute to reduction in quality of life. This emphasises the importance of increasing our understanding of the pathophysiological manifestations of epilepsy/seizures and also to develop new treatment strategies to improve the welfare of epileptic patients.

A 6-month randomised, double-blinded, placebo controlled crossover dietary trial was designed to compare a medium chain triglyceride ketogenic diet (MCTD) to a standardised placebo diet in chronically treated dogs with idiopathic epilepsy. Dogs were fed either MCTD or placebo diet for 3 months followed by a subsequent respective switch of diet for a further 3 months. Clinical and laboratory data and biological samples were collected and analysed.

Seizure frequency was significantly lower when dogs were on MCTD (2.31/month, 0-9.89/month) in comparison to placebo diet (2.67/month, 0.33-22.92/month, p=0.0195) Seizure days were also significantly lower when dogs were on the MCTD (1.63/month, 0-7.58/month) in comparison to placebo diet (1.69/month, 0.33-13.82/month, p=0.0216)[1]. The MCTD also resulted in a significant improvement in the ADHD-related behavioural factor 'chasing' and a reduction in stranger-directed fear (P<0.05) compared with the placebo diet[2]. Metabolomics data modelling reveals significant class separation between diet groups with candidate metabolites of biological interest to be identified with further work.

In conclusion the data shown are in accordance with other studies, showing antiepileptic properties of a MCTD, and provides evidence for the implications of the MCTD as a therapeutic option for treatment of epilepsy. Furthermore the data also supports the supposition that dogs with Idiopathic epilepsy may exhibit behaviours that resemble ADHD symptoms and that the MCTD may be able to improve some of these behaviours. Finally the data suggests that a metabolomics approach may provide further insights into the biological mechanisms involved with the MCTD and epilepsy.

1. Law TH, Davies ESS, Pan Y, Zanghi B, Volk HA. A randomised trial of a medium-chain TAG diet as treatment for dogs with idiopathic epilepsy. The British Journal of Nutrition. 2015; 1438-1447. doi:10.1017/S000711451500313X
2. Packer RMA, Law TH, Davies ESS, Zanghi B, Pan Y, Volk HA. Effects of a ketogenic diet on ADHD-like behaviour in dogs with idiopathic epilepsy. Epilepsy and Behaviour. 2016; 62-68. doi:10.1016/j.yebeh.2015.11.014

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Impact of Foot and Mouth disease on smallholders of the Andean region: implications for surveillance and progressive control

Georgina Limon, Jonathan Rushton, Javier Guitian

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Foot and Mouth Disease (FMD) is considered one of the most economically relevant transboundary animal diseases, mainly because of its trade impact. However, FMD impact and benefits derived for its control might be less obvious for smallholders, where production is mainly focus on subsistence or local trade on small scale. In recent years international programs for FMD control, in resource scare settings, have been justified as a means to improve smallholders' food security, yet the potential benefits on smallholder food security remains unclear.

The overall aim of this project is to develop tools to assess the impact of livestock diseases on smallholders of the Andean region, and to propose strategies for the surveillance and control of FMD; which are non-detrimental for the livelihoods of smallholders.

A mixed methods approach, based on the join collection, analysis and interpretation of quantitative and qualitative data, was used to investigate factors influencing reporting livestock diseases among smallholders in Bolivia. Lessons learnt from this study were used to design the main field study, which involved three Andean countries (Bolivia, Peru and Ecuador) and was carried out in the context of a transnational FMD control programme in the region. A total of 632 smallholders were interviewed. Quantitative and qualitative information collected were analysed, at the household level, using multivariate analysis and thematic analysis respectively in order to: (i) describe smallholders' profiles, (ii) conduct a systematic and comprehensive investigation of smallholders' food security and (iii) develop a system dynamics model to assess the economic impact of controlling FMD identifying leverage points detrimental for smallholders.

Results on the food security assessment and preliminary results from the economic impact component will be presented. The methodological approach presented could be used by policy makers and researchers involved in design and implementation of animal disease control programmes involving smallholders.

The effects of *Enterococcus faecium* NCIMB 10415/SF 68 in the gastrointestinal tracts of healthy dogs

L. Matthewman

Abstract

Probiotics have been shown to reduce the severity and duration of acute multi-causal diarrhoea. *Enterococcus faecium* NCIMB 10415 (SF68) is the only probiotic bacterium licensed for treatment of dogs in Europe. Little is known about the dynamics of this bacterium in the gastrointestinal tract of normal dogs or dogs with acute diarrhoea, nor anything about the possible effects it might have on the microbiota. Synbiotic D-C, a product containing this strain as well as prebiotic additive has been used in a trial to treat acute diarrhoea following doxorubicin therapy and it was also given to normal (control) dogs.

Materials and Methods

Twelve dogs were fed Synbiotic D-C for 14 days and faecal samples were collected on days 0, 4, 10, 14 and day 18 which was 4 days after feeding of the probiotic stopped. DNA was extracted from the faecal samples and submitted for Illumina sequencing and the microbiotas of these samples was analysed using the QIIME bioinformatics pipeline. A qPCR was developed to quantify the bacteria in the faecal samples.

Results

The probiotic bacteria was detectable by qPCR whilst the dogs were fed Synbiotic D-C but the amount declined and was not detectable in some dogs after feeding stopped. There was no effect of the probiotic bacteria on the microbiota of these dogs in terms of species composition or species diversity. A cladogram of the microbiota of these dogs compiled from the data of the samples taken on days 0, 14 and 18 revealed that the only difference between these days was more *Enterococcus faecium* present in the microbiota samples taken on day 14.

Conclusions

These data suggest that *Enterococcus faecium* NCIMB 10415 transiently colonises the gastrointestinal tract of healthy dogs but that it does not affect the microbiota.

* This abstract was accepted although it was submitted after the deadline

The epidemiology of bovine tuberculosis at a wildlife-livestock interface in Uganda

NV Meunier, R Kock, R White

The epidemiology and control of bovine tuberculosis (BTB) is complex, most notably in systems containing multiple host species. In the Queen Elizabeth National Park (QENP) area of western Uganda, there is close interaction between wildlife, livestock and humans, with shared use of grazing and water resources. Current knowledge of the disease burden in this area is limited and the transmission features of the system are poorly understood. The aim of this project was to further understand the epidemiology of *Mycobacterium bovis* at the wildlife-livestock interface around QENP focusing on cattle and African buffalo. Multiple methods were employed including face-to-face interviews, paper surveys, abattoir sampling, and infection testing in the species of interest. BTB was not generally considered an important disease to livestock owners, either due to low disease levels or farmers being unaware of the impact on production in comparison to other diseases. Individual cattle BTB prevalence was low but roughly a quarter of herds showed at least one reactor in the study area. Buffalo BTB infection levels have not increased since 1997 but infection has been detected in the south of QENP for the first time. Although wildlife and cattle regularly shared grazing and watering areas, they seldom came into contact close enough for aerosol transmission of BTB. Between species infection transmission is therefore likely to be by indirect or non-respiratory routes, which is suspected to be an infrequent mechanism of transmission of BTB. Occasional cross-species spillover of infection is possible and the interaction of multiple wildlife species needs further investigation. Controlling the interface between wildlife and cattle in a situation where eradication is not being considered, may have little impact on BTB disease control in cattle.

The application of marketing methodology to the advocacy of on-farm biosecurity: Targeted strategies

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This study proposes a two-stage application of how social science methodology, namely cluster analysis and targeted strategies, can be applied to assist the advocacy of on-farm biosecurity. A semi-structured survey of 303 broiler farmers was conducted in Taiwan to collect farm biosecurity information. It was found that there was attitude-behaviour inconsistency which resulted in compromised biosecurity practices at the farm level. Using categorical principal components analysis and two-stage cluster analysis, two types of cluster were identified: (1) attitude clusters based on 15 variables indicated that despite recognising the importance of biosecurity measures, these farmers tended not to translate their knowledge into the relevant biosecurity actions; (2) behaviour clusters characterised by 30 variables represented that farmers who, despite not having a better attitude towards the importance of biosecurity, still took desirable biosecurity actions. Following this analysis, the study applied the concept of targeted strategies of consumer marketing to veterinary medicine for the advocacy of on-farm biosecurity by identifying five target clusters based on individual farmers' biosecurity attitudes and behaviours. Based on the clusters, relevant targeted strategies were planned, including targeted communication, resource supply, knowledge delivering, policy instruments and enforcement activities. These findings suggest a failure of effective policy implementation as a result of the government placing most effort on providing knowledge (based on the knowledge-attitude-behaviour model) and enforcement of policy (based on the counter-attitudinal advocacy model). Inappropriate application of behavioural change theory will not result in the desired biosecurity behaviours amongst many farmers and may instead induce cognitive dissonance amongst groups of farmers. Targeted strategies will be useful for the development of biosecurity policies in Taiwan's poultry farms.

Who's at risk: Heterogeneities in Individuals' Risk of Tuberculosos in Wild Meerkat Groups

ST. Patterson

Background: Tuberculosis (TB) is an important disease of wildlife, livestock, and humans worldwide, but long-term datasets describing this condition are rare. A population of meerkats (Suricata suricatta) in South Africa's Kalahari Desert are infected with Mycobacterium suricattae, a novel strain of TB causing fatal disease in this group-living species.

Objective: To identify characteristics of individuals and groups at highest risk of developing TB. Methods: We conducted a retrospective cohort study based on a unique, long-term life history dataset that included over 2000 individually-identified animals and spanned 14 years, following on from the population's first TB diagnosis in 2001. Time-dependant Cox regression was used to examine the potential influence of risk factors on development of terminal disease due to TB.

Results: Disease affected 144 individuals in 27 of 73 social groups, in 12 of the 14 study years (incidence rate 3.78 cases/100 study years). Age had the greatest effect on individual-level disease outcome, a hazard ratio of 4.7 (95%CI: 1.92-11.53, p<0.001) for meerkats aged 24-48 months compared to animals aged <24 months, and a hazard ratio of 9.36 (3.34-26.25, p<0.001) for animals >48 months. A history of TB in the group increased the hazard by a factor of 4.29 (2.00-9.17, p<0.001). Immigrations of new group members in the previous year increased the group-level hazard by a factor of 3.00 (1.23-7.34, p=0.016). There was weaker evidence of an environmental effect with a hazard ratio for low rainfall (<200 mm) years of 2.28 (0.91-5.72, p=0.079).

Conclusions: This analysis of this unusually detailed long-term dataset from a wildlife population has advanced our knowledge of individual- and group-level risk factors for TB. Our findings corroborate and build upon knowledge of TB in other species such as European badgers (Meles meles) and Brushtail possums (Trichosurus vulpecula), and hence may assist management of this currently intractable disease.

Effects of Glucagon-Like Peptide-1 Receptor Agonists on bone blow flow and architecture in diabetic mice

M. Pereira

Type 2 diabetes mellitus prevalence increases with age and the disease predisposes to increased risk of fractures and skeletal complications. One possible contributor to skeletal weakening in diabetes is a decline in blood supply. We hypothesised that bone blood flow is impaired in diabetic mice and that chronic administration of Glucagon-Like Peptide-1 Receptor Agonists (GLP-1RA) can increase blood flow to bone, thereby stimulating bone formation and improving bone architecture.

Nine weeks old male diabetic (db/db) and control mice were daily injected subcutaneously for 28 days with saline or the GLP-1RA Exenatide (Ex-4) (10µg/kg/d) (n=10/group). The effect of Ex-4 on hind limb perfusion was measured by laser Doppler imaging. Tibial bone architecture was imaged by micro-CT ex-vivo.

Diabetic mice had -40% lower bone blood flow than control mice (P<0.0001) at baseline. Ex-4 acutely increased tibial blood flow in diabetic mice from 15min of injection to a maximum of 25% increase compared to saline (P<0.0001). Similarly, blood flow was increased with Ex-4 in control mice but at a lower extend than in diabetic mice (+20%, P<0.05). No chronic effect of Ex-4 was shown when blood flow was monitored after the last injection.

Diabetic mice have lower trabecular bone mass compared to controls, due to decreases in trabecular number and thickness. They also exhibit impaired bone connectivity, structure and cortical bone geometry. Ex-4 treatment increased trabecular bone volume (+49%, P<0.01), thickness (+8%, P<0.01) and number (+38%, P<0.01) in diabetic but not in control mice. Connectivity and structure were also improved as shown by decreased trabecular pattern factor (-29%, P<0.0001) and structure model index (-11%, P<0.01).

In conclusion, our results suggest that diabetic mice have lower blood flow and impaired skeletal structure and that Ex-4 exert a bone anabolic action in diabetic mice that could be in part due to its increased skeletal perfusion.

Use of Functional Electrical Stimulation to re-animate the larynx in Recurrent Laryngeal Neuropathy

Justin Perkins, Norm Ducharme and Alan Wilson

Functional electrical stimulation (FES) is a technique for the treatment of lower motor neuron injury. FES can have a marked positive influence on patients, even after chronic denervation. FES of the intrinsic laryngeal musculature has had limited studies performed in both human beings and in animal models. In a small human clinical trial, an external laryngeal pacemaker implant significantly increased the magnitude of laryngeal opening, although the technology to make an implanted pacemaker was lacking at that time.

The aims of this study are to develop new techniques to assess the function and morphology of laryngeal muscles during FES and to investigate the effects of different stimulation patterns during FES of the PCA in horses with naturally occurring RLN. The main aim for this thesis is to compare implanted horses on 3% and 5% daily activation for relatively short term stimulation duration (6 weeks). Development of FES techniques in horses with RLN would have international impact on equine welfare as well as reducing the significant economic loss to the equine industry.

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The isolation and identification of potential vaccine antigens against *Dermanyssus gallinae*, the poultry red mite

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The poultry red mite (PRM), *Dermanyssus gallinae*, is the most economically important ectoparasite affecting laying hens throughout the world. Control of PRM through the use of acaricides is no longer completely effective due to high levels of parasite genetic resistance to currently used compounds. Development of alternative control strategies is urgently required, including the development of effective vaccines. Based on the successful use of the BM86 gut protein in the commercially successful cattle tick vaccine, TickGARD® we aim to identify and characterise potential vaccine antigens that are expressed within the gut of the PRM. Starting with homogenised mites, we tested a series of different protein fractionation methods, resulting in the reliable production of a 'membrane protein enriched' fraction. Examination of this fraction by 2D LC-MS resulted in the identification of peptides derived from >1500 contigs of a PRM transcriptome library (Illumina 100bp paired-end sequencing: 36Gb sequence from 36 billion reads: assembled into 200K contigs). 54% of the peptides appear to be shared with other mite species whilst 34% appear to be unique to *D. gallinae*.

The membrane protein enriched fraction was used to select mite-specific antibodies from a phagemid antibody library (Tomlinson J library, 1.37 x 10^8 phage, MRC, Cambridge) via three rounds of biopanning. We have identified ~400 monoclonal phages of which 32 bind highly to mite protein in ELISA experiments and show specific binding patterns to mite tissues in immunohistochemistry experiments. Sixteen monoclonals bind specifically to gut sections and will be utilized in future experiments to identify immunogenic gut proteins.

Regulation of chorionic girdle development and Equine Chorionic Gonadotrophin

J.E. Read

During preimplantation equine conceptus development, chorionic girdle trophoblasts become terminally differentiated and initiate production of equine chorionic gonadotrophin (eCG). eCG is a heterodimeric glycoprotein crucial in early pregnancy, for maintenance of the corpus luteum and progesterone production. However, very little is known about regulation of trophoblast differentiation or eCG production.

This study aims to increase our knowledge of trophoblast differentiation and transcriptional regulation of eCG by (i) understanding the role of Glial Cells missing 1 (GCM1) in regulation of eCG β-subunit (*eCGβ)* (ii) identifying novel transcription factors that may regulate *eCGβ*. (iii) investigating signalling pathways induced during trophoblast differentiation.

GCM1 mRNA and protein was expressed in day 34 chorionic girdle and correlated with *eCGβ* expression (R^2=0.52). Through creation of truncated lengths of the *eCGβ* promoter and co-transfections, GCM1 was shown to drive the *eCGβ* promoter, acting on two of four predicted GCM1 binding sites.

Microarray analysis highlighted dynamic changes in gene expression specific to the chorionic girdle (ChG) between days 27-34 of pregnancy. Multiplex qRT-PCR was used to validate microarray data. From a gene list (Fc>2, P<0.05, ChG, D31 vs D27), 7 transcription factors correlated with *eCGβ* expression (R^2>0.5) and had predicted binding sites in the promoter. Through co-transfections it was demonstrated that both Ets like factor 5 (ELF5) and Creb 3 like factor 4 (CREB3L4) can drive activity of the *eCGβ* promoter.

A number of signalling pathways changed in activity in the girdle between days 27 and 34 of pregnancy. Activation of the ERK/MAPK pathway was confirmed through protein analysis, with p44/42 ERK becoming phosphorylated at day 30 of pregnancy. Scratch assay analysis demonstrated that equine trophoblast motility was promoted by activation of the cAMP pathway.

A greater understanding of the regulation of trophoblast differentiation and of *eCGβ* production offers an opportunity to better understand miss-regulation may occur in early pregnancy loss in mares. Here we confirm a role for GCM1 and identify novel candidate transcription factors that may play a role in eCG regulation. We also identify signalling pathways that may promote terminal differentiation of chorionic girdle trophoblasts.

Gait dynamics in spiders

<u>Michelle A. Reeve</u>, Stephen Hailes (UCL), Monica A. Daley

Spiders are very agile animals, able to move over many types of terrain, from flat horizontal surfaces to obstacle-ridden landscapes. They can even move effectively in these situations after legs have been lost via a self-amputation defence process known as autotomy. Spiders are very diverse, with different families adapted to different tasks such as fast overground running, building webs, and jumping. Here, I compare the running movements of two different species of spider. Wolf spiders (*Pardosa amentata*; n=8, body mass 34.3 ± 9.8 mg [mean ± s.d.]) are hunters, and run through typically grassy habitats to stalk and pounce on prey. In contrast, orb-weavers (*Araneus diadematus*; n=9, body mass 51.2 ± 25.9 mg [mean ± s.d.]) are primarily adapted to building and walking on webs, using these to catch prey. In this talk I will describe the main differences in overground locomotion between these two distinct species. By filming spiders running across a flat arena at 1000 Hz, we measured and calculated gait dynamics such as speed range, foot slippage, duty factor and relative leg phases. Studying the movement of spiders, which are well adapted for moving over obstacles, can inform the design of new legged robots. These bio-inspired robots could have controllers especially designed to mimic that of a spider, enabling the robot to recover from obstacles and leg damage in a similar way. The uses of a bio-inspired eight-legged robot are twofold: firstly, to test biological hypotheses that are difficult or unethical to carry out on live animals; and secondly, to use as a rescue robot in the emergency services.

Multidisciplinary approach for improving diagnosis and treatment of canine neuropathic pain

Sandra Sanchis-Mora

Chiari-like malformation and syringomyelia (CM/SM) is a canine disease complex that causes neuropathic pain (NeP). Validating canine spontaneous models of NeP is of great value to represent its clinical complexity that cannot be captured with experimental models. This project aimed to (i) characterise canine CM/SM; (ii) validate a NeP assessment toolbox; (iii) evaluate the efficacy of pregabalin in the treatment of NeP in dogs; (iv) validate CM/SM as a spontaneous model of canine NeP.

From the overall canine England population, the period prevalence of CM/SM was low. Pain was the most common associated clinical sign. Lack of insurance was a cause of underestimation of the prevalence and to the misdiagnosis of this painful condition.

A sensory testing protocol was developed to identify canine somatosensory abnormalities. Response to mechanical and thermal stimuli were assessed in healthy dogs. The protocol was well tolerated showing substantial reliability. The protocol was applied to Cavalier King Charles Spaniels (CKCS) with NeP associated with CM/SM and asymptomatic CKCS. Symptomatic dogs presented cold allodynia and mechanical hyperaesthesia compared to asymptomatic dogs. The second tool of NeP assessment was an owner questionnaire. After development and validation, correlation with other chronic pain assessment tools is being studied.

Using the NeP toolbox, the efficacy of pregabalin was evaluated in a placebo-controlled double-blinded crossover clinical trial on CKCS with NeP. We expect pregabalin to have a significant effect on the indicators of the NeP toolbox.

The NeP toolbox identified somatosensory disturbances and behaviours associated with NeP and evaluated the efficacy of drugs suggested to treat NeP. CM/SM is therefore a good model for canine NeP to explore new drug development. Canine CM/SM should be considered as a disorder of major welfare impact. With these tools and the data provided we can improve the awareness, diagnosis and treatment of dogs suffering NeP from CM/SM.

Identification and characterisation of myeloid cell populations in porcine tonsils

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Porcine tonsils are the portal of entry of several pathogens of the pig, including classical swine fever (CSFV). Cells of the myeloid lineage are targets for the virus and include macrophages and dendritic cells; cells responsible for the uptake of pathogens, antigen recognition and orchestrating the adaptive immune response. The aim of this project is to identify the myeloid cell populations found in the porcine tonsil and study their interaction with pathogenic and attenuated CSFV strains.

In order to further define myeloid cells resident in the porcine tonsil we firstly excluded all cells expressing typical lymphoid lineage markers from dissociated tonsillar cells. The remaining cells were then stained with an 8 colour flow cytometry panel including Live/Dead, lineage antibodies (CD21, CD8a, CD3, IgM), CD4, CD14, MHC-II, CD172a, CD163 and CADm1. The 5 cell populations identified were putatively assigned as, macrophages, pDCs (lin$^-$CD172a^{low}, MHC-II$^{neg/low}$, CD4+), cDC1 (lin$^-$,CD172a^{low} MHC-II^{high} CADm1^{high}), cDC2 (lin$^-$CD172a^{high}, MHC-II^{high}, CADm1$^{low/neg}$) and CD14+ DCs (lin$^-$ CD172a^{high}, MHC-II^{high}, CD14^{low}). Across all 5 subsets, freshly isolated cells expressed both MHC class II and CD80/86, which increased dramatically following 4 hour culture, with highest levels consistently associated with cDC1, cDC2 and CD14+ DCs. CD14+ DCs (and macrophages) also showed the highest antigen processing capacity. Myeloid cell subsets showed differential ability to respond to different TLR agonists; macrophages and CD14+ DCs responded primarily to LPS (TLR4 agonist). cDC1 DCs secreted TNF-α following Poly I:C (TLR3 agonist) and IL-12 after CpG (TLR9 agonist) stimulation indicating parallels between their pig and human cDC1 counterparts. Finally, infection of the cell populations with CSFV *in vitro* showed similar levels of infection with virulent strain Alfort, but much higher levels of infection in pDCs associated with the attenuated strain (C strain).

Characterisation of DC/Macrophage subsets will provide a tool to study their interaction with CSFV and critically to target emerging vaccines to specific DC populations of the host.

Estimation and monitoring of the frequency of selected endemic diseases and conditions of dairy cows in Great Britain

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Background

Sustainability of dairy farms relies on good cattle health and welfare. In order to help guide disease control efforts, and protect public health through production of safe food, accurate and up-to-date information on cattle health at national level is needed. In Great Britain, there is currently no centralized recording system for recording of such data.

Aim

The aim of this project was to provide new information on disease frequency at national level and to generate new tools that could inform and enhance existing disease surveillance strategies.

Methods

Important health issues were identified and ranked in a workshop with representatives from the dairy industry and cattle health experts. Usefulness of the recording systems to provide good quality information at national level was evaluated. A nationwide field study of dairy farms was carried out to estimate the frequency of selected diseases and conditions of dairy cows and to identify their potential risk factors. As part of this study, farmers' perception on disease occurrence was also collected.

Results

The evaluated systems showed considerable differences in terms of their coverage, design, implementation and objectives. Although the existing systems provide important information for individual farmers, usefulness of such information at national level is limited. The results of the field study showed that the studied diseases and conditions are spread widely and mostly occurring with high frequency. Based on farmers' perception, mastitis, lameness and fertility remain the most important conditions of dairy cows.

Conclusions

No accurate and up-to-date information on frequency of the studied diseases is currently available at national level. Considering the level at which the studied diseases are currently present and their economic implications, controlling such diseases is of great importance to individual farmers, dairy industry and the general public. Information on disease frequency can provide valuable baseline data for monitoring of the effectiveness of the current and future control programmes at national level.

Development of novel biotheraputics for Staphylococcus aureus infection

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Staphylococcus (S.) aureus is responsible for many skin and soft tissue infections across a broad range of species. One of particular note is bovine mastitis, a disease resulting the largest costs to the dairy industry, for which S. aureus is one of the major causative agents. A contributory factor to these losses is the recurring nature of infection due to the inability of the host to elicit an effective immune response to S. aureus. The main treatment method is antibiotics, however, a 50% treatment failure rate has been reported. The use of such antibiotics within agriculture is being discouraged due to concerns over the progress of antibiotic resistance and drug residues in food products. These factors compound the need for the development of novel treatment strategies.

The use of artificial opsonins could be one such treatment strategy using the carbohydrate binding region of C-type lectin pattern recognition receptors and the Fc portion of IgG1. The premise being that lectin fragment of the fusion protein binds the carbohydrates present in the surface structures of S. aureus and the Fc part binds Fc receptors present on immune cells. The resulting signalling would initiate phagocytosis, reactive oxygen and cytokine release and consequently enhance the clearance of the bacteria.

The first fusion protein created utilised the carbohydrate recognition domain (CRD) of mannose binding lectin (MBL), a secreted C-type lectin involved in the lectin complement pathway. Little or no binding of the fusion protein to either mannose or S. aureus was seen. This is possibly due to the affinity of the CRD as the native form of MBL has a complex quaternary structure containing up to 15 CRD regions. A second fusion protein has been created using the CRD and neck region of the C-type lectin Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN). Once the ability of this protein to bind S. aureus has been confirmed the effect on the uptake and killing of the bacteria by phagocytes will be determined in vitro.

POSTER ABSTRACTS

POSTER 1: Kavin Abelak

Molecular Determinants of Fatty Acid Metabolism by Cytochrome P450 Enzymes

Abstract:
Cytochrome P450 (CYP) enzymes are mono-oxygenases responsible for the metabolism of many endogenous and exogenous chemicals. Biochemical and structural analysis of CYPs indicate their catalytic centres contain an iron atom within a porphyrin ring. Polyunsaturated fatty acids, such as arachidonic acid (ARA), are metabolised by particular subfamilies of CYPs with epoxygenase and lipoxygenase activities. CYP2J2 is one such epoxygenase and the products of its metabolism of ARA are vasodilators, pro-angiogenic, and are anti-inflammatory. CYP2J2 metabolises ARA into specific stereo- and regio- isomers but, due to the absence of a crystal structure, the precise mechanism(s) is not fully understood.

The aim of the current work was to understand the recognition of ARA by CYP2J2 using computational modelling. Using a selection of high quality crystal structures in complex with ligands of similar size to ARA, a set of models was generated and evaluated. Through a series of molecular dynamics (MD) steps in AMBER, the chosen homology model was equilibrated in solvent to mimic the cellular environment. Following computational docking of ARA into the active site of CYP2J2 using AutoDOCK VINA, the complex was further equilibrated by MD. Analysis of the data showed a number of residues within the active site cavity providing favourable energetic contributions to hold ARA in place over the heme. An *in vitro* mutational investigation is planned to determine the influence of each of those residues on the catalysis of ARA.

POSTER 2: Sarah Allen

Equine Vetcompass: Generating Evidence Through Practice-Based Equine Health Surveillance

Abstract:
The application of evidence-based veterinary medicine (EBVM) in equine practice is seriously hampered by the limited body of reliable information, even for the most common conditions. The shortage of accurate prevalence data stems from a comprehensive lack of endemic disease monitoring in the general horse population. Robust, clinically-relevant disease statistics can be generated through the analysis of electronic patient records (EPRs), from first-opinion veterinary practice; an approach which has been successfully used to study a number of small animal disorders in the UK. By implementing a secure protocol for the automated collection of anonymised EPR data in a group of equine practices, this project aims to establish an efficient and sustainable system for the surveillance of UK horse health.

At each episode of care, the attending veterinary surgeon will tag the EPR with the standardised summary term (VeNom code) that best describes the reason for examination. Anonymised EPR data, including patient demographics, free- text clinical notes and VeNom codes, will then be extracted and transferred to a central database for analysis. Initial analyses will estimate the prevalence of, and identify risk factors for, the most common conditions observed in equine practice.

First-opinion electronic clinical records are an underused source of equine disease information. By facilitating the use of these valuable data for epidemiological research, Equine VetCompass has the potential to produce accurate disease statistics, representative of the national horse population. This information can be used to establish health benchmarks, set research priorities and improve healthcare provisions through the more targeted training of veterinary professionals. Moreover, Equine VetCompass studies can be designed to fill existing knowledge gaps, with the reliable disease information generated capable of supporting EBVM in equine practice.

POSTER 3: Alana Burrell

Evidence that the refractile bodies of *Eimeria* converge following invasion

Authors: Alana Burrell[1,2], Sue Vaughan[1], Virginia Marugan-Hernandez[2], Fiona Tomley[2]
[1] Dpt of Biological and Medical Sciences, Oxford Brookes University; [2] Dpt of Pathology and Pathogen Biology, The Royal Veterinary College, London

Abstract:
Since the 1960's, ultrastructural studies have played an important role in advancing our understanding of the complex biology and life-cycles of *Eimeria* and other coccidian parasites. With recent advances in high resolution instrumentation there is now an opportunity to expand on this body of existing knowledge. Using a combination of three dimensional imaging techniques alongside high resolution electron microscopy, we aim to produce quantitative data on the internal anatomy of different developmental stages of *Eimeria tenella* as it infects and develops within target host cells. By serially sectioning the surface of a sample and using scanning electron microscopy to image each freshly sectioned surface, accurate three dimensional reconstructions can be produced at high resolution. Using the invasive sporozoite stage we are currently defining the numbers, volumes and spatial relationships between different classes of internal organelles. We have also been able to compare these findings to those of sporozoites which have recently invaded a mammalian cell. One of the most striking observations was that the refractile bodies retain the same total volume despite reducing in number from two to one supporting the hypothesis that there is an amalgamation of the material rather than a loss of one of the bodies. Future work will use advanced imaging techniques, along with our initial quantitative datasets, to further examine how the ultrastructure of both the parasite and host cell changes throughout its life cycle. We also plan to assess the impact of targeted disruptions, for example to parasite and/or host biochemical pathways that are implicated in parasite attachment, invasion and intracellular replication.

POSTER 4: Marianne Carson

Behavioural adaptations of poultry production stakeholders to disease outbreaks and different control policies in Bangladesh

Authors: Marianne Carson, Dirk Pfeiffer and Guillaume Fournié (RVC, UK), Chris Desmond (University of the Witwatersrand, South Africa), Ahasanul Hoque (Chittagong Veterinary and Animal Sciences University, Bangladesh)

Abstract:
Many zoonotic livestock diseases are intrinsically linked to human activities, yet the role of human behaviour is an often marginalised aspect of disease prevention and control. This project will seek to understand the behaviour and decision-making processes of Bangladeshi poultry production stakeholders during disease outbreaks and under different control policies. Poultry farmers and traders may change their behaviour during outbreaks to avoid economic losses, for example poultry farmers may sell sick birds at a reduced price before the flock dies or is culled. Studying these kinds of behaviours will help improve our understanding of how they influence disease spread and help in the development of context-appropriate policies and interventions.

To achieve this, a novel approach involving behavioural economic games will be used to look at decision-making in different groups within the poultry trade network. Behavioural games are tools for experimentally investigating specific types of behaviours or decision-making processes. They usually involve multiple players and participation involves the possibility of real losses or gains in the form of money or other types of incentives. For this study experimental games will be developed and carefully adapted to the specific local setting. To assist in this process, interviews and surveys will be used to develop locally produced epidemiological scenarios relevant to the study population which will then become part of the games themselves.

This project will contribute to the advancement of more efficient and cost-effective approaches to dealing with complex diseases situations in Bangladesh. The behaviour data could also be used to improve epidemiological models. Finally, one broader outcome will be the novel methodology itself, which is intended to be a practical tool that can be used in other settings and for other diseases as part of a holistic approach to understanding animal and public health issues.

POSTER 5: Wan-Ching Cheng

An exploration of the molecular pathways driving left ventricular hypertrophy in cats

Abstract:
Left ventricular hypertrophy (LVH) is characterized by thickening of the wall of the left ventricle and can be the result of both physiological and pathological stimuli. These stimuli instigate LVH through separate but interconnected signaling pathways. A greater understanding of these pathways and in particular how they interconnect, may lead to treatments for pathological LVH such as hypertrophic cardiomyopathy (HCM); the most common cardiac disease of cats. Previous work at the RVC has shown that cats with hypersomatotropism (HS) develop LVH which resolves following treatment, akin to the reversible physiological LVH seen in the athletic heart. This is in contrast to the non-reversible pathological LVH seen in HCM. This project aimsI to explore the different signaling pathways driving LVH in these two naturally occurring models of feline cardiac hypertrophy. This will be achieved using, immunohistochemistry(IHC) and Western blot (WB) analysis on left ventricular tissue from cats carefully phenotyped in the clinic. In collaboration with the University of Birmingham the activity of signaling pathways traditionally associated with physiological and pathological hypertrophy will be assess in 3 groups of cats (control, HCM and HS). Results will be compared with a histopathological score of severity of pathological change consisting of myofibre disarray, interstitial fibrosis and intramural vasculopathy.

Hypothesis 1. LVH in cats with HCM is predominantly the result of pathological signalling, whereas LVH associated with HS is predominantly the result of physiological signalling.
Hypothesis 2. Prolonged physiological signalling may induce myocardial changes characteristic of pathological signalling due to crosstalk between the pathways.
Results: To date appropriate tissue has been sectioned and good cross reactivity on IHC has been shown for a number of key signaling proteins on feline tissue.
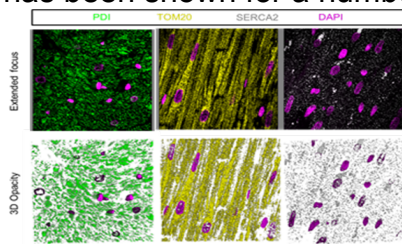
Immunohistochemical staining of myocardium from a cat with HCM showing cross-reactivity of mouse and human antibodies against common intracellular proteins (PDI = ER marker; Tom20 = mitochondria; SERCA2; SR Ca2+ ATPase)
We also aim to explore the role of cardiac progenitor cells in these two forms of hypertrophy. Previous published work has shown that the number of c-kit (CD117) positive CD45 negative cardiac progenitor cells increases in atrial and ventricular tissue of adult cats subjected to experimental catecholamine induced cardiomyopathy. It remains unclear whether these progenitor cells develop into predominantly cardiomyocytes or vascular endothelium. We aim to quantify the number of progenitor cells in the 3 cohorts of cats previously described and explore potential correlations with other endothelial markers, for instance, PECAM1 and VWF.
Hypothesis:
Cardiac progenitor cells will be increased in cat with HS and cats with HCM
Results:
To date we have shown that the antibody used in the previous published study also works on our sectioned tissue.

POSTER 6: Bhagyalakshmi Chengat Prakashbabu

Food safety interventions in peri-urban dairy farms in West Africa (Senegal & Togo)

Abstract:
From tapeworm to *E coli,* food borne illnesses pose a major threat to the global population. A recent report by the World Health Organisation (WHO) titled 'WHO estimates of foodborne illness, 2009-2015' indicates that the burden of food borne illness is highest in Africa, affecting all age groups but posing a significant threat to children under 5 years. Peri-urban farms in West Africa provide food security and economic support to the ever growing urban population. However, they also act as a source of milk borne pathogens. Raw milk consumption and dairy products made from raw milk are integral part of their food culture. This PhD aims risk based prioritisation of milk borne pathogens, design and test of food safety interventions. Cities will be selected in each of the two countries. Cross-sectional studies will be designed to understand milk and milk products value chain. Risky practices that compromise food safety at different stages of production will identified through questionnaire based cross-sectional studies. Based on the data collected qualitative risk analysis will be conducted to prioritise pathogens based on their risk of human infection. Milk samples will be collected from selected peri-urban farms and tested for prioritised pathogens. Food safety interventions in pre-harvest, harvest and post-harvest levels will be designed and evaluated using simulation modelling or system dynamics. The outcomes of the study are expected to contribute to capacity building, improve food safety, decrease incidence of food borne illnesses and hence improve public health.

POSTER 7: Megan Conroy

Chronic Kidney Disease in cats in the UK: Prevalence and descriptive study.

Authors: Megan Conroy, Dave Brodbelt, Yu-Mei Change, Dan O'Neill, Jonathan Elliott.

Abstract:
Chronic Kidney Disease (CKD) is commonly diagnosed in cats. Little is known about diagnosis and treatment of CKD in cats in the UK. This study aims to estimate the prevalence of CKD in veterinary attending cats in the UK and describe diagnostic procedures and treatments prescribed.

All cats that presented to 244 practices in the VetCompass programme between January 2012 and December 2013 were included in the study. Electronic patient records were searched for key terms and treatments associated with CKD diagnosis to identify potential CKD cases. A random sample of 20% of the potential cases was reviewed in detail to identify CKD cases. Data were extracted for demographics, diagnosis and treatment. Prevalence was estimated adjusting for the sampling method.

Of 353,488 cats presented to the participating practices, 11,768 potential cases were identified. From the potential cases reviewed in detail, 625 new cases and 338 pre-existing cases were identified. Apparent prevalence was estimated at 1.2% (95% Confidence Interval (CI) 1.1% - 1.3%). An increase in prevalence was found in older cats (<9 years 0.1% (95% CI 0.1% - 0.2%), ≥ 9 years 3.6% (95% CI 3.3% - 3.8%)). Most cats (66.6%) were presented by their owners due to clinical signs associated with CKD. Combined biochemistry and urinalysis following clinical exam was the most common method of diagnosis (52.6%). An IRIS CKD stage was recorded in 19.8% of cases. A commercial 'Renal' diet was the most frequently prescribed treatment (63.2%).

Prevalence of CKD was found to increase in older cats, with just over 1 in 30 cats ≥ 9 years being diagnosed with CKD. Improved awareness of the frequency of CKD, why owners are presenting cats to veterinarians prior to diagnosis of CKD and how veterinarians are diagnosing and treating CKD will allow targeted education to owners and veterinarians.

POSTER 8: Laura Craighead

Economics of Brucellosis control in West and Central Africa

Supervisors: Prof Jonathan Rushton, Prof Javier Guitian

Abstract

Africa has the highest rate of urbanization, with this brings a shift from populations of rural subsistence farmers to urban populations reliant on expanding and changing food systems. At the same time sub Saharan countries particularly in West and Central Africa suffer the largest burdens from neglected endemic zoonotic diseases such as brucellosis. A bacterial disease that is transmitted to humans through consumption of contaminated dairy products or by direct contact with infected animals, brucellosis causes flu-like symptoms and chronic debilitating illness. In livestock, brucellosis decreases productivity by causing abortions, reducing fertility and decreasing milk yield.

Brucellosis has been successfully controlled and eliminated in many countries by means of vaccination and test and slaughter progammes. In the changing farming systems seen in West and Central Africa a successful control program within the peri-urban dairy industry would prove hugely beneficial for both human health and the dairy industry in terms of productivity and profitability.

This project aims to characterize the peri-urban dairy sector and assess the impact brucellosis has at present. Mapping out the industry using value chain frameworks will allow identification of future patterns within the industry and important stakeholders associated with disease control. Using participatory epidemiology methods to investigate behavior of the various stakeholders will allow identification of appropriate vaccination delivery methods within this setting. During the project a vaccination programme will be piloted in selected areas using a case control study to compare the effectiveness of selected vaccine delivery methods.

Given the scale of the burden of brucellosis, the outputs of the research will hopefully help to inform policy makers on appropriate and effective control methods not only in the study countries but in other settings with similar changing dairy industries.

POSTER 9: Alana Dowling

Evaluation of a yeast-based BVDV vaccine approach

Abstract:
Bovine viral diarrhoea virus (BVDV) is a pestivirus that affects cattle globally and is widely recognised as one of the most economically important infectious diseases of cattle[1]. In addition to biosecurity measurements, utilisation of vaccines provides cost-effective systems to control infectious animal diseases, reduce production costs and enhance welfare of animals. Conventional cattle vaccines are parenterally administered killed or modified live vaccine (MLV) preparations. In the case of BVD, vaccines have been used for decades but BVD is still endemic in many counties worldwide [3, 4]. The investigation of novel approaches to vaccination aims to tackle future challenges in disease control.

The yeast-based vaccine approach has been extensively studied, in species such as *Saccharomyces cerevisiae* and *Pichia pastoris*, as a potential live antigen delivery system that betters conventional vaccines[5]. Advantages include the fact that yeast-based vaccines are non-pathogenic to animals, easily administered, simple and cost effective to produce, do not require adjuvants, are stable, and induce both mucosal and systemic immune responses when delivered orally or intranasally[6-9]. In the present project, the yeast-based vaccine consists of whole, freeze dried *S. cerevisiae* that express cell surface bound BVDV antigens. Pattern recognition receptors (PRRs) expressed on innate immune cells within the intestinal mucosa trigger the detection and uptake of yeast particles. This uptake of yeast initiates a cascade of events including maturation of DCs, pro-inflammatory cytokine secretion and induction of an antigen-specific cell mediated immune response [10-12].

We hypothesise that oral application of the yeast-based BVDV vaccine will induce a systemic and mucosal immune response to subsequent challenge. To test this hypothesis, we aim to orally vaccinate calves against BVDV using the yeast-based BVDV vaccine. To validate this aim we intend to assess our recombinant-yeast in the vicinity of M-cells/GALT structures, assess cytotoxic T cell response, conduct a dose-finding experiment, and carry out a proof-of-concept challenge experiment.

1. *EU thematic network on control of bovine viral diarrhoea virus (BVDV)*. 2006.
2. Walz, P.H., et al., *Control of Bovine Viral Diarrhea Virus in Ruminants.* Journal of Veterinary Internal Medicine, 2010. **24**(3): p. 476-486.
3. Newcomer, B.W., et al., *Efficacy of bovine viral diarrhea virus vaccination to prevent reproductive disease: A meta-analysis.* Theriogenology, 2015. **83**(3): p. 360-365. e1.
4. Moennig, V. and P. Becher, *Pestivirus control programs: how far have we come and where are we going?* Anim Health Res Rev, 2015. **16**(1): p. 83-7.
5. Bill, R.M., *Recombinant protein subunit vaccine synthesis in microbes: a role for yeast?* Journal of Pharmacy and Pharmacology, 2015. **67**(3): p. 319-328.
6. Bernstein, M.B., et al., *Recombinant Saccharomyces cerevisiae (yeast-CEA) as a potent activator of murine dendritic cells.* Vaccine, 2008. **26**(4): p. 509-21.
7. Stubbs, A.C., et al., *Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity.* Nature medicine, 2001. **7**(5): p. 625-629.
8. Haller, A.A., et al., *Whole recombinant yeast-based immunotherapy induces potent T cell responses targeting HCV NS3 and Core proteins.* Vaccine, 2007. **25**(8): p. 1452-63.
9. Barron, M.A., et al., *Human dendritic cell interactions with whole recombinant yeast: implications for HIV-1 vaccine development.* Journal of clinical immunology, 2006. **26**(3): p. 251-264.
10. Rizzetto, L., et al., *Systems biology of host-mycobiota interactions: dissecting Dectin-1 and Dectin-2 signalling in immune cells with DC-ATLAS.* Immunobiology, 2013. **218**(11): p. 1428-37.
11. Romani, L., *Immunity to fungal infections.* Nat Rev Immunol, 2004. **4**(1): p. 1-23.
12. Ardiani, A., J.P. Higgins, and J.W. Hodge, *Vaccines based on whole recombinant Saccharomyces cerevisiae cells.* FEMS Yeast Res, 2010. **10**(8): p. 1060-9.

POSTER 10: Enrico Eberhard

A robotic foot driven by musculoskeletal simulations of frog jumping – towards more realistic environmental interactions in forward modelling

Supervisor: Christopher Richards

Abstract:

Forward dynamic simulation is a powerful tool for predicting joint trajectories under hypothetical physiological conditions. Contrary to experimentation, simulation parameters such as muscle activation and segment lengths can be varied easily to address questions on evolution or performance. However, these simulations often lack realistic substrate interactions. Ground reaction force (GRF) is the fundamental driver of terrestrial locomotion, yet most simulations use simplified contact models that neglect the rich interplay between limb and substrate. A new method is proposed to generate physical GRF from real substrates, using a robotic frog foot operating in parallel with a real-time simulation: the robotic segment is driven by simulated joint moments, while the resulting robotic deflection updates the motion of the virtual body to simulate jumping. A preliminary system with a single robotic joint and a simplified simulation model was developed to demonstrate the feasibility of hardware driven by virtual dynamics. The current prototype works toward the long-term goal to build a three joint robot capable of translating across a substrate according to a simulated trajectory. The method will determine how various substrates influence GRF characteristics, internal dynamics and emergent limb kinematics to provide new insight into stability and control of frog jumps on compliant substrates. Moreover, this project steps towards a larger ambition of interfacing simulations with real-world inputs and outputs to realistically and representatively bring hypothetical biomechanics to life.

<u>**POSTER 11:**</u> **Catherine Gaskin**

Do cellular cholesterol concentrations affect macrophage responses to amyloid-β peptides?

Abstract:

By 2050 it is estimated that 131.5 million people will be affected worldwide by Alzheimer's disease (AD) (World Alzheimer's Report-2015). Plaques, comprised of amyloid beta ($A\beta_{1-42}$) peptides accumulate in the brains of AD patients which are surrounded by activated microglia (brain macrophages). $A\beta_{1-42}$ aggregates stimulate macrophages to produce cytokines including Tumour Necrosis factor alpha, Interleukin-1 Beta and Interleukin-6. Observational studies have made links with increased cholesterol levels and the onset of AD. In particular, the genetic risk relating to *APOE4*, a lipid/cholesterol transporter within the brain has been associated with an increased risk of developing AD. Our objectives are to identify whether manipulating cell membrane cholesterol, in particular lipid rafts, result in an altered cytokine response to $A\beta_{1-42}$. The addition of $A\beta_{1-42}$ to RAW264 cells (a macrophage line) caused an increase in membrane cholesterol, an increase in lipid rafts and a release of pro-inflammatory cytokines. Pre-treatment with cholesteryl ester hydrolase (CEH) inhibitors caused an increase in cholesterol stores and a reduction in cytokine production. Pre-treatment with Acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitors, which inhibit the esterification of cholesterol, gave an increase of lipid raft-associated cholesterol and also enhanced the $A\beta_{1-42}$ induced cytokine production. The immunosuppression caused by CEH inhibitors was associated with inhibition of $A\beta_{1-42}$ induced translocation of the signalling protein Toll like receptor-4 (TLR-4) to cholesterol-sensitive lipid rafts. We concluded that stimulation of macrophages with $A\beta_{1-42}$ induced cholesterol recruitment to lipid rafts. CEH inhibitors which prevent cholesterol recruitment to lipid rafts, reduced the level of $A\beta_{1-42}$-induced cytokine production. To identify that this mechanism also occurs in the brain parenchyma we are now using a zebrafish model to measure the concentration of microglia in the forebrain upon injection with $A\beta_{1-42}$. We shall also investigate whether pre-treatments with our CEH/ACAT inhibitors result in variations in microglia numbers within the zebrafish forebrain.

POSTER 12: Sophia Gonzalez-Medina

Equine atypical myopathy: Modelling the disease in vitro and determining field risk factors

Authors: S. Gonzalez-Medina, R.J. Piercy

Equine atypical myopathy (AM) is an acute and severe rhabdomyolysis of grazing horses caused by the toxic metabolite of hypoglycin A (HGA), methylenecyclopropyl acetic acid (MCPA), contained in seeds and seedlings of some Acer trees. The incidence of the disease seems to have increased in in recent years: most affected horses die or are euthanased due to severe impairment of cardiac and respiratory muscles. Despite unproven and limited efficacies, treatments are chosen, based on postulated support of mitochondrial metabolism and antioxidant administration. However, mortality remains high and severe pain and secondary morbidities have significant welfare implications for horses and profound emotional impacts on horse owners.

The aims of this project are to stablish a cellular-based model of AM that helps to understand the pathophysiological mechanism of disease in order to evaluate and develop new therapeutical options for the treatment of the disease. In addition, we are seeking to determine field risk factors associated with HGA content in seeds and seedling of A. pseudoplatanus.

Development of a Cell-based model for AM
a) Determination of MCPA toxicity in murine myoblasts (C2C12) and horse fibroblasts and myoblasts using different assays for evaluation of mitochondrial metabolism (MTT assay), cytotoxicity (LDH assay) and cell death (Propidium iodine).
b) Evaluation of mitochondrial dysfunction of cells treated with MCPA
c) Effect of several treatments in MCPA treated cells

Determination of field-associated risk factors
d) Validation of LC-MS techniques for hypoglycin A measurement in plant extracts.
e) Sample side calculation for field studies regarding two experimental conditions:
   -HGA contained in seeds and leaves of tress affected and unaffected by Tar Spot
   -Changes in HGA concentration in Acer pseudoplatanus seeds during maduration

Development of a clinically applicable test for diagnostic confirmation of AM

Preliminary toxicity assay results have shown that horse fibroblasts are more sensitive to MCPA than murine myoblasts. Validation curves have been produced for quantitation of HGA in plant extracts and sample size for field studies calculated.

<u>POSTER 13:</u> **Russell Guire**

An objective investigation looking at saddle kinematics and its effect on equine locomotion in sound horses.

Authors: R. Guire[1,2], A. Barstow[1], M. Fisher[3], H. Mathie[4], B. Benson[5], R. Weller[1], T. Pfau[1]

Abstract:
Saddle fit and positioning is vital for optimizing equine spinal kinematics and providing the rider with stability and support to execute their desired movements.
Objectives: To evaluate the effect which a saddle positioned LATERALLY to the vertebrae has on equine kinematics compared to a CENTRALLY positioned saddle.
Methods: Quantitative gait study in a convenience sample. Seven horses (1.63-1.80m; 6-12 years), displaying saddle SLIP, assessed for lameness by a veterinarian. Saddle fit was assessed and adjusted by 3 Society of Master Saddlers Qualified Saddle Fitters. Horses were equipped with 4 inertial sensors (poll, sacrum, tuber coxae), twenty-four joint centre markers were used to capture 2D kinematics (Quintic Biomechanics) with 2 synchronized high speed cameras (spatial resolution 1300x400, 400 fps at 10m distance) recording left and right lateral views. A Pliance (Novel) pressure mat determined peak pressures (3 repeats of 2 strides), maximum carpal and tarsal flexion, fetlock hyperextension and peak pressures were compared between LATERALLY and CENTRALLY positioned saddles. Horses were warmed up following a standard 20 minute protocol and then trotted (rising trot) and cantered by their associated rider on both reins (3 repeats). Data was collected with SLIP, then saddles were adjusted/rebalanced (STRAIGHT) using Prolite shims and differences in range of movement (ROM) and peak pressure were assessed with a paired T-test (P<0.05).
Results: Saddle slip grade using a paired T-test (P<0.05) was significantly smaller for CENTRAL (graded 0-5, mean 0.78) versus LATERAL (mean 3.32, P=0.003). In left rein trot, larger values were found for STRAIGHT for mediolateral ROM of poll, sacrum and right tuber coxae (all P <0.04) and craniocaudal ROM of left tuber coxae (P=0.001). In right rein trot, smaller values were found in the left tuber coxae (P=0.02) when CENTRAL. In left rein canter, smaller sacrum and right tuber coxae ROM were found for CENTRAL (P<0.04) and no difference on the right rein. In trot a saddle LATERALLY positioned left on the right rein, a decrease in (P=0.05) left forelimb fetlock hyperextension (lateral (L) 112.1°, central 109.7°) and when LATERALLY positioned to the right on the left rein, a decrease (P<0.0001) in right forelimb fetlock hyperextension (lateral (R) 109.5°, central 106.6°) was found compared to CENTRAL. A decrease (P=0.02) in maximum force (lateral (R) 586 N, central 507 N) in the rear, right panel was found when CENTRAL on the left rein. In canter no differences were found (P>0.06) between CENTRAL and LATERAL positioning in maximum carpal, tarsal flexion or fetlock hyperextension. In left-lead canter with a saddle positioned LATERALLY left, a decrease (P=0.009) in peak pressure was observed in the rear right panel when CENTRAL (Lateral (L) 29.61 kPa, central 26.16 kPa). In right-lead canter with saddle positioned LATERALLY left, a decrease (P=0.05) was observed in the rear left panel when CENTRAL (lateral (L) 28.16 kPa, central27.33 kPa).
Conclusions: In trot, a saddle positioned CENTRALLY significantly increases mediolateral ROM on the rein coinciding with the direction of SLIP and increased loading along with reduced asymmetric pressures under the saddle. In canter, reduced ROM was observed with a CENTRAL saddle along with reduced asymmetric saddle pressures.

POSTER 14: Caroline Hodgkinson

Optimising equine biosecurity awareness and practices to reduce the welfare impact of infectious disease

Authors: C.R. Hodgkinson1&2, J. Slater2, M.L. Brennan3 and J.L. Ireland1
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3The University of Nottingham, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD

Abstract:
Equine infectious diseases represent a major welfare concern and result in considerable financial losses. Biosecurity encompasses a range of hygiene and management practices designed to reduce the introduction of infectious agents and to control their spread within populations. Voluntary biosecurity recommendations are available within the UK Thoroughbred industry, but the extent to which existing guidelines are utilised in the non-racing population is currently unknown. This project aims to determine horse owner awareness and perceptions of biosecurity, describe facilities available on non-racing equestrian premises and establish expert consensus to inform improved uptake of practical, effective evidence-based biosecurity recommendations. This project will be conducted in three stages. Phase one will describe biosecurity practices and facilities, knowledge and perceptions of equine biosecurity in a cross-section of randomly selected horse owners, evaluated via a postal questionnaire. Questionnaires will be collected from approximately 1,000 owners. Horse owners will be recruited to the study using multiple sources: registered clients of participating equine veterinary practices; County Council licensed riding establishments; and members of the British Horse Society and British Equestrian Federation. In Phase two, approximately 150 respondents will receive a site visit to allow a detailed evaluation of biosecurity facilities and spatial layout of the premises. In Phase three, the Delphi method will be used to gather expert opinions and consensus to produce evidence-based owner and veterinary biosecurity guidelines. Owner perceptions of biosecurity will be assessed using behaviour change theories to identify the most effective methods to deliver targeted owner education. Through the development of practical evidence-based biosecurity guidelines and an improved understanding of horse owners' biosecurity decision-making process, this study will ensure that biosecurity recommendations are more likely to be sustainably adopted by a larger proportion of owners. In turn, this will reduce this spread of infectious disease and improve equine health and welfare.

POSTER 15: Hannah Holt

Milk-borne public health hazards in the Indian Punjab: a preliminary risk assessment

India is now the world's leading milk producer following the establishment of dairy cooperatives for the procurement of milk, linking rural producers to urban markets. Within India, the Punjab state has the highest milk availability per capita and cattle and buffalo are owned by the majority of rural households. Rapid growth of the Punjabi dairy industry presents many opportunities for development such as access to export markets. However, there are also threats, including increased disease risk attributed to higher livestock densities, trade and use of exotic breeds. In addition to causing financial losses to the industry, diseases with zoonotic potential pose a direct threat to consumer safety. The aim of this work was to develop a qualitative risk assessment framework to assess the risk of biological and chemical public health hazards, including brucellosis, arising from the consumption of cattle and buffalo milk and dairy products produced in the Indian Punjab.

The preliminary risk assessment was based on the principles of the Codex Alimentarius Commission. Data for the risk assessment was assimilated from a structured literature review specific to the Punjab study area and expert elicitation via key-informant interviews and an expert opinion workshop. The information gathered to inform different stages of the assessment included: the dairy industry and supply chains in the Punjab, the presence and epidemiology of milkborne hazards and the processing and consumption practices for milk and dairy products. Exposure was assessed according to the occurrence in livestock (estimates from the literature & expert opinion) or likelihood of cross-contamination and the growth and survival dynamics of the hazards. Disability-adjusted life years (DALY's) per 1000 cases were estimated to classify the severity of biological hazards.

Milk in the formal channel is routinely pasteurised and assumed sufficient to destroy microbiological hazards, however Escherichia coli and Staphylococcus aureus have been detected in pasteurised milk in the Punjab. Pasteurisation and safety checks are not performed in the informal sector through which ~70% of milk is marketed. However, as milk is always boiled before consumption as liquid milk and usually (but not always) before processing into other products, the risk of all biological hazards was considered negligible – low. The risk of Cryptosporidium parvum, E. coli (e.g. EHEC, STEC) and Staphylococcus aureus were considered low. Brucella abortus, Campylobacter spp., Leptospira spp., Listeria monocytogenes, Mycobacterium bovis, Salmonella spp. and Streptococcus spp. were considered very low, and the rest of the identified hazards (Bacillus cereus, Coxiella burnetti, and Streptococcus spp.) considered negligible. Chemical hazards are not destroyed by pasteurisation or heat treatment by the consumer. Therefore the risk of pesticides and adulterates were considered non-negligible and antimicrobials and aflatoxins were considered low. Although there is limited data to quantify their dose-response effects there are concerns that long-term exposure to pesticides and aflatoxins may be carcinogenic and antimicrobial residues in foodstuffs may have a role in the development of antimicrobial resistant infections in humans. These hazards are considered a priority hazards and there is a need for the identification of risk management strategies to reduce the presence of these hazards in the final product in order to ensure consumer safety.

POSTER 16: Natalie Moyen

Transmission of avian influenza viruses through live bird trade networks in Bangladesh

PhD supervisors: Guillaume Fournié, Dirk Pfeiffer.

Abstract:
Avian influenza (AI) is endemic in Bangladesh and 90% of poultry and poultry products transit through live bird markets (LBMs), which play a major role in spreading viruses through human and poultry populations. Bangladesh's fast growing economy and increasing consumer demand are expected to drive an increase in poultry production and volumes of live birds being traded through LBMs.

The aim of this study is to assess the potential of the network of contacts between poultry farms and LBMs resulting from live poultry trade in Bangladesh to maintain and spread poultry viruses, and to identify suitable targets for control and surveillance programs in order to limit the likelihood of an outbreak, or reduce its' impact if it were to happen.

A cross-sectional questionnaire survey was carried out from April to December 2015, in 3 different sub-districts of Bangladesh in order to collect information on trading practices: number and type of traded poultry, poultry's origin and destination, surplus management and prices.

In total, more than 2,000 interviews were carried out in 159 LBMs, with various types of actors involved in poultry trading, through LBMs, from the farm gate to the end-consumer (market managers, middlemen, stall-holders, feed-dealers). Preliminary observations of the data collected showed that the poultry trading network seems to vary greatly according to the type of poultry traded (local, crossbreed and broiler chickens, ducks) and the period of the year (festivals, seasons).

The next steps of the project will consist in describing the poultry transaction chains according to poultry type. Secondly, a description of the network of contacts created through poultry trading movements will be carried out. Thirdly, mathematical models will be used to assess the potential for amplification and dissemination of AI in the network. Finally, using models, I will assess the impact of targeted control and surveillance methods.

POSTER 17: Muneeswaran Narayanan Selvaraj

Development of reverse genetics technique to rescue lineage IV PPR viruses from c-DNA clones and their use to study early pathogenesis of PPR

Abstract:
Across the developing world, Peste des petits ruminants virus (PPRV), places a huge disease burden on agriculture, in particular affecting small ruminant production. PPRV is a single stranded, non-segmented, negative-sense RNA virus belonging to the genus *morbillivirus* under the family *Paramyxoviridae*. Although two safe and efficacious live attenuated vaccines are available for PPR control; similar to the Plowright rinderpest vaccine, as observed during the global rinderpest eradication campaign, current PPR vaccines and serological tests do not enable differentiation between naturally infected and vaccinated animals (DIVA). Therefore the main aim of this PhD project is to develop DIVA vaccines using reverse genetics for the available vaccine strains. A secondary problem with current PPR vaccines is the slow generation of neutralising antibodies, although the vaccine is very effective at producing long term immunity. Neutralising antibodies can only be detected 2 to 3 weeks post vaccination. The natural route of PPRV infection is usually through the respiratory tract. Recently, it has been shown that PPRV infects susceptible hosts more efficiently when administered intranasally, in comparison to the subcutaneous route. Therefore, it is essential to compare the efficacy of vaccines using both routes of administration. Furthermore, the pathogenesis of PPRV, particularly the primary replication site in the pre-viremic stage is not very clear. Contrary to previous reports it has been recently shown that the initial site of virus replication is not within the epithelial cells of respiratory mucosa. Therefore, it is essential to identify the primary site of virus replication for PPRV infection, so that it can be targeted during vaccination to generate a quick and appropriate immune response. Within the first six months of this PhD, the Sungri-96 PPR vaccine strain has been rescued from a full length cDNA clone and currently attempts are being made to develop it as a DIVA vaccine.

<u>**POSTER 18:**</u> **Jose Novo Matos**

Transient myocardial thickening in cats associated with heart failure

Authors: J. Novo Matos[1,2], N. Pereira[2], T. Glaus[2], L. Wilkie[1], K. Borgeat[1,3], J. Loureiro[4], J. Silva[4], V. Law[1], D.J. Connolly[1], A. Kranjc[2], V. Luis Fuentes[1]
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Abstract:
Cats with hypertrophic cardiomyopathy (HCM) and heart failure (CHF) have a poor prognosis. Nevertheless there are anecdotal observations of cats with HCM that show normalization of left ventricular wall thickness (LVWT) and an excellent prognosis. Transient increase in LVWT mimicking HCM has been described in acute myocarditis in humans.

We aimed to identify and describe clinical characteristics in cats with transient myocardial thickening and CHF (TMT+CHF). We hypothesized that TMT+CHF occurs mainly in young cats with a history of an antecedent event (e.g. neutering).

Clinical records at a single center were searched for TMT+CHF cases and a control group of cats matched for clinical signs with persistent increased LVWT and CHF (HCM+CHF); and also at multiple centers for TMT+CHF cases. TMT+CHF was defined as initial LVWT ≥6mm accompanied by signs of CHF with subsequent decrease in wall thickness to <5.5mm. Data are reported as median [range] or mean (95%CI).

Case-control study: 6 TMT+CHF and 6 HCM+CHF cats were identified. TMT+CHF were younger (1.7 [0.4-4.0] years) than HCM+CHF cats (10.2 [8.2-12.6] years, p=0.004). At presentation LVWT was similar in TMT+CHF (6.7 [6.1-8.2] mm) and HCM+CHF groups (7.7 [6.0-9.8] mm, p=0.297), but TMT+CHF cats had thinner LVWT at final echo (5.0 mm [3.7-5.3] vs 7.2 mm [5.5-8.3], p=0.004). Left atrium/aorta (LA/Ao) was larger in the HCM+CHF group at presentation (LA/Ao 2.9 [1.98-3.23] vs 2 [1.52-2.3], p=0.024) and increased in size over time, (to 3.1 [2.37-3.83] vs a decrease to 1.4 [1.26-1.56] in TMT+CHF cats, p=0.006). 83.3% of TMT+CHF cats had antecedent events vs 50% in the HCM+CHF group (p=0.545). CHF relapsed in all HCM+CHF cats but in no TMT+CHF cats.

Multicenter study: 20 TMT+CHF cases were enrolled. 75% had antecedent events. LVWT decreased over 3.4 (95%CI 1.9-4.8) months from 6.8mm [6.0-9.7] to 4.8mm [2.8-5.3], (p<0.0001) and LA/Ao also decreased (from 1.8 [1.5-2.30] to 1.4 [1.3-1.7], p= 0.001). cTnI was elevated at presentation and normalised once myocardial thickening resolved (cTnI 3.7ng/ml [0.05-63.8] vs 0.01ng/ml [0.0-0.34]; p=0.018). Therapy was discontinued in 18/20 TMT+CHF cats at 4.6 (CI 2.3-6.9) months after presentation and all cats remained asymptomatic.

TMT+CHF affects younger cats and antecedent events are common, however no clinical or echocardiographic characteristics at presentation differentiated the two groups. TMT is a cause of CHF in cats but has a better prognosis than HCM+CHF.

POSTER 19: Ashley Otter

Understanding the regulation of cytochrome P450s: core enzymes involved in Mycobacteria metabolism

Authors: Ashley D. Otter[1] and Sharon Kendall[1]
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Abstract:
Mycobacterium is a highly diverse genus of bacteria, including the pathogens *M. tuberculosis* and *M. bovis*, the cause of human and bovine tuberculosis. It has been estimated that *M. tuberculosis* (Mtb) is the biggest cause of death by infectious disease worldwide, with an estimated 1/3 of the world's population infected with Mtb and in 2014 alone, there were 9.6 million active TB infections worldwide leading to 1.5 million deaths[1]. *M. bovis* causes zoonotic infections in humans and more significantly, tuberculosis in cattle, an ever growing problem within the UK and other countries. *M. bovis* has been estimated to have cost the tax payer £500 million in the past decade, likely to increase to £1 billion within the next decade if infections continue to rise[2].

Though *M. tuberculosis* is well studied, the metabolism of the bacterium is not well understood, with only limited research available. Cytochrome P450s (CYPs) are absent in numerous bacteria, but *Mycobacterium* is an exception, with *M. tuberculosis* and *M. bovis* having 20 and 17 CYPs, respectively[3]. CYPs play a key role in metabolism and have been found to be attractive drug targets, due to their importance in bacterial survival[3,4], but little is known about their regulation.

A large number of CYPs are associated with TetR family of transcriptional regulators (TFTRs), a highly abundant group of transcriptional regulators in *M. tuberculosis*, though their function is unknown. To further understand TFTRs and their role in CYP regulation, a range of bioinformatics and molecular biology techniques are to be performed, including recombinant TFTR expression and purification, ligand-binding studies and electrophoretic mobility shift assays, generation of TFTR mutants and RNA-Seq. To date, three TFTRs hypothesised in regulating CYPs have been successfully cloned and purified.

References:

1) World Health Organization (2012). Global tuberculosis report 2012. Geneva, Switzerland. http://www.who.int/tb/publications/global_report/
2) Department for Environment Food and Rural Affairs (2014). The Strategy for achieving Officially Bovine Tuberculosis Free status for England. London, UK. www.defra.gov.uk
3) Munro, a W., McLean, K. J., Marshall, K. R., Warman, a J., Lewis, G., Roitel, O., Leys, D. (2003). Cytochromes P450: novel drug targets in the war against multidrug-resistant Mycobacterium tuberculosis. *Biochemical Society Transactions*, **31**:625–630.
4) McLean KJ, Marshall KR, Richmond A, Hunter IS, Fowler K, Kieser T, Gurcha SS, Besra GS, Munro AW (2002). Azole antifungals are potent inhibitors of cytochrome P450 mono-oxygenases and bacterial growth in mycobacteria and streptomycetes. *Microbiology*. **148**:2937-49.

POSTER 20: Carlos Rebelo

Evaluation of the Current Electrical Stunning Systems used by Small Scale Producer/Processors for Turkeys, Ducks and Geese in the UK

Authors: CB Rebelo, S Abeyesinghe and T Gibson
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Abstract:
There is limited information on current poultry and waterfowl stunning/slaughter practices in small scale processing plants, farms and abattoirs in the United Kingdom (UK). Specifically, no detailed evidence on the techniques, equipment and settings used for the non-waterbath electrical stunning of turkeys, ducks and geese currently exists. Stunning poultry before slaughter is a legal requirement (EU 1099/2009, WATOK 2015) adopted to safeguard animal welfare. In Europe the minimum electrical currents for head-only electrical stunning are 400 and 240 mA for turkeys and chickens, respectively, but there are no minimum required currents for ducks or geese, and little published peer-reviewed information on the effectiveness of stunning in these species on which to base legislation or practice standards.

To address the abovementioned deficits, face-to-face interviews with staff/managers at UK poultry and waterfowl processing plants were undertaken to investigate current practices and issues experienced by the industry. Interviewees were recruited by industry contacts. One-to-one structured interviews averaging thirty minutes were conducted, focusing on technical aspects of the stunning parameters used, technical details of the handling and restraining methods, meat quality of slaughtered birds, as well as the industry attitudes towards improving the current system.

The three most commonly used electrical stunning systems determined by the interviews (head-only/head-to-body/head-to-shackle) and electrode types will be tested in laboratory conditions mirroring the processes performed in commercial operations, and assessed (using electrophysiology and behaviour) in terms of the period of induced unconsciousness/insensibility and the time to complete recovery from the stun. This will allow the detailed assessment of the currently used methods in terms of performance and welfare. Information from the interviews will also help identify potential areas for refinement and development of existing and new stunning systems, and to improve animal welfare and effectiveness whilst minimising negative impacts on product quality, processing costs and ensuring industry relevance.

POSTER 21: Dominique Riddell

Multiplex quantitative gene expression analysis in skeletal muscle for evaluating neuromuscular disease and performance in horses

Authors: Dominique O Riddell, Dr Sam M Mirczuk, Dr Rob C Fowkes, Professor Richard J Piercy.

Abstract:

Background
Comprehensive evaluation of the disease- and performance-related aspects of racehorse skeletal muscle is reliant on evaluating anatomical traits, plasma biochemistry or histology. For the latter, a relatively large muscle sample is needed that necessitates up to 2 weeks' convalescence and time off from training programmes. Further, biopsy of small muscles (such as the intrinsic laryngeal muscles) for research can cause iatrogenic damage. A potential alternative analytic method, requiring a far smaller sample, is quantification of expression of selected genes in muscle associated with performance and neuromuscular disease.

Aims
To design, optimise and validate a multiplex, quantitative reverse-transcription, PCR-based method for simultaneous evaluation of expression of up to 18 pre-selected genes in equine muscle samples obtained by micro-biopsy.

Methods
Selected genes include those involved in fibre typing, oxidative capacity, growth, repair, atrophy or inflammation. Muscle samples were obtained from horses immediately post-mortem from the crico-arytenoideus dorsalis and gluteus-medius muscles. Primers were designed and samples tested using the GenomeLab GeXP Genetic analysis system.

Results
mRNA from 15/18 genes was simultaneously detected, however, unsurprisingly, there was marked disparity in relative expression of different genes. Subsequent analysis of primer pairs for each gene individually, revealed that quantification of high-expressing genes in the multiplex, likely inhibited detection of low expressors. Lowering the reverse primer concentration for genes with the highest expression, increased relative fluorescence of genes with a previously low or absent signal (p<0.001). Further examination revealed that splitting the multiplex into 2 assays, might enable optimal analysis.

Conclusions
This multiplex method is feasible, however competition within the reaction inhibits detection of mRNA from low expressing genes. The next step is to test validation by examining repeatability, comparing gene expression profiles of healthy and diseased muscle, and correlating results with conventional methods of analysis, such as histology.

POSTER 22: Hannah Safi

Blood Flow and the Development of Aneurysms: Novel Investigative Measurements

Authors: Hannah Safi, Nathan Phillips, Richard J. Bomphrey

An Abdominal Aortic Aneurysm (AAA) represents a degenerative disease process of the abdominal aorta that leads to a focal dilation and irreversible remodeling of the arterial wall. An AAA is defined as an enlargement of the aorta by at least 1.5 times its original diameter in the maximum transverse or anterior-posterior planes. In the clinical setting, it is a controversial question whether to surgically repair an unruptured AAA. clinicians must decide when the risk of an AAA rupture justifies the risks associated with surgical repair. Yet, at present there is no accepted method to quantify the risk of rupture for individual AAAs. Elective repair decisions are generally founded on the "maximum diameter criterion", typically 5.5 cm. However, this criterion is a general rule-of-thumb and known to be unreliable because there are a significant number of studies (10-24%) that show AAAs smaller than this threshold diameter can also rupture. A biomechanics-based approach to rupture prediction built on computational models can be applicable in the clinical setting. Recent guidelines for treatment of AAAs from the Society for Vascular Surgery suggest computationally acquired rupture predictors need further validation prior to their implementation in a clinical setting. In this current work, we present an emerging technique where simultaneous fluid flow and atrial wall strain measurements are carried out using the methods of Particle Image Velocimetry and Digital Image Correlation respectively. Measurements are tested on a highly simplified silicone AAA model. We demonstrate this combined technique for investigating the fluid-structure interactions between blood flow and vessel wall deformation. Visualising the experimental physical modelling of internal flows and wall surface deformations highlights great potential in validating computational models.

POSTER 23: Joshua Sealy

ZELS-Associated Studentship; Molecular Epidemiology of H9N2 avian influenza viruses in Pakistan

Supervisors: Munir Iqbal (Main supervisor, The Pirbright Institute), Dirk Pfeiffer (RVC), John McCauley (MNIMR), Tahir Yaqub (Pakistan)

Abstract:
Avian influenza viruses (AIV) of the H9N2 subtype are causing devastating losses in poultry in many countries throughout Asia, the Middle East and North Africa including Pakistan. Recent evidence suggests that H9N2 viruses have a particularly high tendency for donating internal genes by reassortment to other co-circulating AIV, and these internal genes may contain molecular markers associated with mammalian transmissibility, thus posing a risk for pandemic emergence of novel human-transmissible genotypes.

To reduce the impact of H9N2 in poultry, several countries employ vaccination, however the efficacy of these programs is compromised by continued rapid adaptive evolution of the virus, and the emergence of variants that are not covered by current vaccine formulations. This project will investigate the epidemiology of H9N2 in Pakistan, a country that has experienced severe outbreaks of H9N2 avian influenza in poultry. This project aims to characterise H9N2 virus diversity in poultry productions systems at varying levels of industrialisation, examine the role of live bird markets in H9N2 virus transmission and to evaluate antigenic diversity and implications for vaccine effectiveness.

POSTER 24: Aarti Singh

Focusing on the mitochondrial expression of TSPO as a marker and promoter of neuroinflammation

Authors: Aarti Singh[1], Kenneth Smith[2], Michelangelo Campanella1[3]

1   Department of Comparative Biomedical Sciences, Royal Veterinary College, London, United Kingdom.
2   Department of Neuroinflammation, University College London, London, United Kingdom.
3   University College London Consortium for Mitochondrial Research, London, United Kingdom.

Abstract:
Translocator Protein (TSPO), previously known as the Peripheral Benzodiazepine Receptor (PBR), is a small (18kDa) 5 transmembrane domain protein that is primarily localized on the outer mitochondrial membrane (OMM). Its reported function lies in the ability to bind to cholesterol and shuttle it across the mitochondrial membrane into the intermembrane space.
TSPO is ubiquitously expressed in peripheral tissues and at particularly low level in the healthy brain. Though, there is a marked upregulation in microglial cells following brain injury, inflammation and in the presence of neurodegeneration. The molecular mechanism underlying the involvement of TSPO in neuroinflammation remains ill-defined even though targeting of the protein is exploited to diagnose inflammatory states of the brain through positron emission topography (PET) scanning of TSPO specific radioligands.

The goal of my project, run in collaboration with GE/Heath Care, is to provide an in vitro pharmacological characterization of the radioligand, GE-180 designed by our Industrial Partner to obtain an adequate profile of its binding capacity to neuroglial cells in neuroinflammatory conditions.
Despite the recurrent upregulation of TSPO in neuroinflammation, the exact function in this context remains devoid of a satisfactory characterization of the underlying pathways and hence of the potential effects mediated by GE-180 as well as the other TSPO ligands used in diagnostic. In the attempt to address this, we made use of the CRISPR/Cas9 genome editing system to target TSPO for knockout (KO) in mouse microglial cell line (BV2) to function as a negative control for TSPO function in vitro. We successfully established a stable TSPO knockout BV2 cell line, validated via biochemical read-out and sequence scrutiny. Via this toll we now plan to perform a number of functional studies by mimicking inflammatory conditions via LPS-based protocols.

Hitherto, our preliminary data suggest that the TSPO KO correlate with a reduced inflammatory phenotype, while parental BV2 show marked upregulation in TSPO signal, confirming a role of TSPO in neuroinflammation.
A major aim now is to identify if neuroinflammation is prior or consequent to TSPO upregulation to better inform a pathophysiological condition common to brain pathology.

POSTER 25: Freija ter Heegde

Role of Piezo2 in the Development of Osteoarthritis and the Associated Pain

Abstract:
Osteoarthritis is a degenerative joint disease that leads to cartilage erosion and joint remodelling which is typically accompanied by chronic pain. The disease currently affects more than 8 million people in UK and is considered the leading cause of invalidity in people above 50 years. We aim to unravel the role of the ion channel Piezo 2 in the development and maintenance of osteoarthritis and its associated pain. Piezos are recently discovered ionic channels that are mechanosensitive. Translation of mechanical stimuli into biological signals is essential for numerous physiological processes, including touch and pain perception. Activation of Piezo2 is involved in both allodynia and hyperalgesia. Interestingly, Piezo2 was also shown to have a role in chondrocytic cell death after injurious mechanical loading. Chondrocytes are the only cells found in cartilage and are responsible for the maintenance of the tissue. Injurious loading is known to damage the cartilage and can, consequently, lead to the onset and progression of osteoarthritis. We will test the hypothesis that Piezo2 expressed in joints is involved in the development and maintenance of osteoarthritis as well as the pain affiliated with the disease. A range of both *in vitro* and *in vivo* methods will be utilized to determine if and at what stage the Piezo2 protein is involved in osteoarthritis and how it contributes to the associated pain. By deciphering the mechanisms by which Piezo2 is involved in this disease we, ultimately, hope to develop novel therapeutic targets for preventing the progression of osteoarthritis and alleviate the associated pain.

POSTER 26: Ross Worrall

Investigating the role of Kit in canine mammary tumours

Background:
Canine mammary tumours (CMT) are the most common tumours in entire bitches, with around half being malignant and higher pathological grades being associated with poorer survival. Recent research has identified KIT, a receptor tyrosine kinase, as a significant predictor of outcome and metastasis in human breast cancer. Preliminary data indicate association of elevated KIT expression with malignancy and increasing grade of malignancy in CMT.

Hypotheses:
- KIT overexpression is a negative prognostic indicator for and contributes to progression of CMT
- KIT activation contributes to CMT progression
- KIT expression in tumours correlates with amplification
- KIT overexpression results in activation of downstream signalling

Objectives:
- Obtain ethical approval for investigation of clinical tissue and data
- Compile and characterise panel of canine mammary tumour cell lines for in vitro investigation
- Establish appropriate cell seeding densities for in vitro assays measured over varying time periods
- Establish conditions for validity of cell viability assessment using Resazurin

Results:
- Ethics approval obtained
- Cell line panel established including a range of canine mammary tumour types
- Seeding densities established
- Demonstrated linear relation of fluorescence intensity (535nM excitation, 590nM emission) with cell number following incubation of cells with Resazurin

Future work:

In confirmed cases of canine mammary carcinoma, to assess
- Associations of KIT expression with survival and metastasis
- KIT amplification (FISH)
- Expression of KIT ligand in tissue

In cell lines,
- examine the native KIT expression, and activation of KIT signalling pathways
- manipulate KIT expression
- stimulate and inhibit KIT pharmacologically
- assess the effect of these interventions on cell phenotype (particularly viability, apoptosis and migration), KIT signalling and downstream gene expression

POSTER 27: **Julia (Ying) Wu**

The Immune Regulatory and Molecular Phenotype of Canine Multicentric B Cell Lymphoma

Abstract:

Lymphomas are one of the most common types of tumours in dogs, and the most prevalent subtype, diffuse large B-cell lymphoma (DLBCL), shows striking similarities to human non-Hodgkin lymphoma. Regulatory T cells (Tregs) are an important component of the tumour microenvironment (TME), which plays a well-established role in tumour progression and immune evasion. Better understanding of this cell population in canine BCL will not only further delineate the biology of the TME, but will also add to our understanding of the disease in both dogs and humans, ultimately leading to the development of novel therapies applicable to both species.

Multi-colour flow cytometry was adopted in this study to phenotype both the lymphoma and Tregs, using four panels of canine-specific and cross-reactive antibodies. All flow cytometric panels were validated and optimised using peripheral blood mononuclear cells from healthy dogs. To date, 24 healthy dogs' blood and, both lymph node and blood samples of 14 dogs presented with lymphadenopathy have been analysed. Combined with contemporaneous cytological review, the 14 diseased dogs' immunophenotype was confirmed, including cases of B cell lymphoma (n=7), T cell lymphoma (n=3), histiocytic sarcoma (n=1), mast cell tumour (n=1) and reactive hyperplasia (n=2). An intriguingly high frequency of Helios expression was observed amongst $CD5^+CD8^+$ T cells in healthy dogs' peripheral blood. No specific pattern of Tregs has been observed in lymphoma cases to date, but sample acquisition is continuing.

Next-generation sequencing (NGS) will be performed to examine the transcriptomic profile of canine DLBCL on FACS™-sorted subpopulations, focusing on infiltrated T cells, myeloid cells and neoplastic B cells. Protocols are currently under optimisation for the preparation of good-quality RNA for sequencing. By means of flow cytometry and NGS, we aim to identify additional regulatory and molecular signatures of predictive impact in canine BCL. Functional assays informed by the results of NGS will follow to investigate the suppressive mechanisms of Tregs and their interactions with neoplastic B cells.