

## CANINE RESEARCH FOUNDATION - FUNDED RESEARCH

The Canine Research Foundation (CRF) is the official vehicle for funding ANKC Ltd research program. The CRF is an independent public charitable trust, originally set up by the Kennel Control Council (now DogsVictoria) in 1992 and funded via puppy registration levies, fund-raising functions, public donations and bequests. Funds are now generated from all ANKC Member Bodies.

Breed Clubs and Breed Councils are invited to suggest research topics for consideration, and each year the CRF calls for applications for grants for research to be conducted by Australian universities.

Applications for grants pass through three review stages:

1. A highly qualified and experienced technical review panel, assessing scientific merit, novelty, potential benefits to canine health and probability of success, with a priority ranking.
2. Review by the Member Bodies' representatives on the ANKC Canine Health Committee, with the benefit of the review panel findings, for their specific input on relevance and priorities.
3. Review by the CRF Trustees to;
  - (a) ensure that applications fit within the Foundation's Trust Deed;
  - (b) collate the findings of the above two review stages and determine overall priorities;
  - (c) award research grants as appropriate within the financial constraints of the CRF.

Since commencement the CRF has funded a over 90 grants for a total funding exceeding \$890,000. This is a sizeable and very significant contribution to improvement of canine health, nationally and internationally. There are five new grants for research commencing in 2013, and continuing funding for two grants awarded in 2012.

The large majority of completed projects have achieved successful outcomes, with results of research disseminated through Australian and international veterinary journals and conferences, with due credit given to CRF for funding. A selection of abstracts of historical projects producing significant results, together with more recent grants, is given below.

### CRF GRANTS

#### SIGNIFICANT GRANTS PRE-2007

##### **Blood Studies**

(Dr. Parry, University of Melbourne)

CRF grants were awarded for research on a series of relatively small but important projects on blood. The projects were:

- Coagulation disorders (1994)
- Coagulation defects caused by snake envenomation (1995)
- Studies on blood donor dogs (1996)
- Plasma products for transfusion (1998)
- Collagen binding assay for von Willebrand disease (1999)
- Factor VII deficiency in dogs (2000).

These all produced valuable research findings, disseminated in many veterinary and dog journals and were key components in the establishment of a specialist referral centre for blood disorders and Australia's first and only canine blood bank. The research on blood donor dogs was the basis of the PhD thesis by Dr. Sato that defined the standard operating procedures and animal welfare factors for a blood bank.

## **Uterine Function and Infertility in the Bitch**

(Drs. Watts, Wright and Lee, University of Melbourne, Vet. Science)

The research commenced in 1995 under a modest CRF grant and developed into a major project with international acclaim. Further grants were awarded and the overall project was completed in 2003. The initial broad aims were to develop new knowledge and skills for the improved diagnosis, treatment and management of infertility in the bitch, and to make this knowledge and skills available to the veterinary profession and to dog breeders. Research in the first stage of the project was included in the work for a PhD thesis for Dr. Watts.

The specific aspects and results of the research work were as follows:

- To develop a reliable test for mis-mating of a bitch.

An accurate, safe and effective procedure for the diagnosis and treatment of mis-mating in the bitch was developed. This involved cannulation of the uterus and the flushing and aspiration of a small volume of saline, followed by microscopic examination of the fluid for the presence of sperm. The test was accurate for 6 days after mating.

- To develop a reliable method of inducing uterine contractions for treatment of uterine infections and to assist in difficult births.

A safe and effective procedure for inducing uterine contractions was developed, using a continuous low dose of prostaglandin for 7 days from a subcutaneous implant.

- To determine the time and hormonal basis of sloughing of the wall of the uterus for the understanding of infertility of bitches with short inter-oestrus intervals, in the treatment of bitches that do not have normal reproductive cycles, and for programs for induction of fertile oestrus in the bitch.

The timing was determined to be at the end of dioestrus and the start of anoestrus. It is related to falling and low plasma progesterone concentrations.

- To fully understand the pathogenesis of the Cystic endometrial hypoplasia (CEH) - pyometra complex. CEH is a thickening and cyst formation of the wall of the uterus. CEH was a common condition that caused infertility and could result in serious life-threatening infection of the uterus. Diagnosis of CEH required a uterine biopsy or detection of cysts using ultrasound, but uterine biopsy was invasive and ultrasound techniques were inaccurate.

A technique was developed that involved visualising the cervix with a rigid endoscope and passing a catheter through the cervix into the uterus. Samples for microbiology and cytology were obtained by the infusion and aspiration of sterile normal saline. It was found that the uterine microflora often reflected the vaginal microflora during pro-oestrus and oestrus, but rarely at other stages of the reproductive cycle. The technique facilitated diagnosis of post-partum metritis, pyometra, endometritis, abortion, retained placenta, postpartum uterine rupture, endometrial sub-involution and misalliance.

Research findings were disseminated as follows:

- "Investigating uterine disease in the bitch: uterine cannulation for cytology, microbiology and hysteroscopy", J.R.Watts and P.J Wright, *Journal of Small Animal Practice* (1995) 36, 201-205.

- “The development and application of transcervical cannulation of the uterus for cytology and microbiology in the bitch”, J.R.Watts, P.J Wright, C.S.Lee and K.G.Whithear, presented at the 27th. annual conference of the Australian Society for Reproductive Biology, Melbourne (1995)
- “Uterine, cervical and vaginal microflora of the normal bitch throughout the reproductive cycle”, J.R.Watts, P.J.Wright and K.C.Whithear, Journal of Small Animal Practice (1996) 37, 54-60.
- “The use of transcervical uterine cannulation for the diagnosis of uterine disorders in the bitch”, J.R.Watts, P.J.Wright and C.S.Lee, presented at the 13th. International Congress on Animal Reproduction, Sydney (1996).
- “Endometritis in the bitch”, J.R.Watts, presented at the 13th. International Congress on Animal Reproduction, Sydney (1996).
- “Diagnosis and treatment of infertility in the bitch – progress, problems and perspective”, J.R.Watts, P.J.Wright and C.S.Lee, presented at satellite symposium on reproduction in dogs, cats and exotic carnivores at 13th. International Congress on Animal Reproduction, Sydney (1996)
- “New techniques using transcervical uterine cannulation for the diagnosis of uterine disorders in bitches”, J.R.Watts, presented at the 3rd. International Symposium on reproduction in dogs, cats and other carnivores, Netherlands (1996)
- “New techniques using transcervical uterine cannulation for the diagnosis of uterine disorders in bitches”, J.R.Watts, P.J Wright, C.S.Lee and K.G.Whithear, Journal of Reproduction and Fertility, Supplement 1997;51:283-93
- “Transcervical uterine Cannulation for the investigation of reproductive tract function in the bitch” J.R.Watts, presented at Cornell University,(1996)
- “Transcervical uterine Cannulation for the investigation of reproductive tract function in the bitch” J.R.Watts, presented to the Swedish Society for Small Animal Reproduction, Uppsala (1996)
- “Endometrial cytology of the normal bitch throughout the reproductive cycle”, J.R.Watts, P.J.Wright and C.S.Lee, Journal of Small Animal Practice (1998) 39, 2-9.

### **Estimating canine blood cortisol levels**

(Investigator: Dr. P.Irwin: Veterinary Clinical Science, Murdoch University)

Serum cortisol values are used to determine whether dogs have significant adrenal axis illness, current adrenal function, and to monitor therapy for adrenal illness. Absolute serum cortisol values are crucial when determining whether to proceed to therapy for adrenal disease in dogs. The RIA method is the accepted standard, but chemiluminescence is the method used in most commercial assays. Experience has shown serious variation in results between the two methods.

Research findings are that although RIA and chemiluminescence cortisol concentrations appear highly correlated, a significant difference may exist when measuring cortisol concentrations less than 100 nmol/L in stored canine sera. The results of chemiluminescence cortisol assays should be interpreted with caution unless the specific assay method in the laboratory has been adequately validated in normal dogs and dogs with hyperadrenocorticism.

- "Comparison of radioimmunoassay and chemiluminescent assay methods to estimate canine blood cortisol concentrations." Russell NJ, Foster S, Clark P, Robertson ID, Lewis D, Irwin PJ. Aust Vet J. 2007 Dec;85(12):487-94.

## **Disease Gene Mapping**

(Investigator: Dr. A.Wilton: University of NSW)

Neuronal Ceroid lipofuscinosis (NCL) in Border Collies was the main focus of this research over a number of years. The funds from CRF grants, the Batten's Disease Association and Border Collie Clubs allowed attraction of extra funding from a government SPIRT grant.

Development of the canine genome map allowed a change in approach to the identification of the NCL gene. Regions of conservation between dog and human gene maps and the knowledge of 7 different genes that can cause similar symptoms in humans provided a good starting point to identify candidates for the NCL gene. Genetic markers (microsatellites) close to the presumed site of these candidate genes were typed in the available Border Collie NCL- affected pedigrees to indicate whether one of the homologues of the human NCL genes is a likely cause of NCL in Borders.

The causative gene for Border Collie NCL was identified - it is the same mutation as one causing Batten's disease in humans. A direct genetic test for Border Collie NCL is now available and has been used very successfully in selection of mating pairs in avoiding production of affected animals.

- "A mutation in canine CLN5 causes neuronal ceroid lipofuscinosis in Border collie dogs." Melville SA, Wilson CL, Chiang CS, Studdert VP, Lingaas F, Wilton AN. *Genomics*. 2005 Sep;86(3):287-94.

## **Effect of oral ursodeoxycholic acid on bile acid tolerance tests in healthy dogs**

(Investigator: Dr L.Abraham: Veterinary Science, University of Melbourne)

Significant hepatic disease is characterised by normal or elevated preprandial (before feeding) and elevated postprandial (after feeding) serum bile acid (SBA) concentrations. Although SBA values cannot differentiate between liver diseases, elevated SBA values may be seen with such disorders as cirrhosis, chronic hepatitis, hepatic necrosis, neoplasia, portosystemic vascular anomalies and cholestasis. Bile acids are synthesised from cholesterol exclusively in the liver and measurement of SBA concentration is routinely used as a diagnostic test for liver function. The bile acid tolerance test consists of oral administration of bile acids with measurement of SBA concentrations to monitor liver function in maintaining SBA levels.

The major bile acids are cytotoxic at sustained elevated levels, possibly leading to complete hepatic failure. Ursodeoxycholic acid (UDCA) is a bile acid usually present in trace amounts only and is not cytotoxic in many species. In human patients, UDCA has been used to treat various chronic cholestatic disorders, but there are few reports of its use in dogs and no clinical trials have been reported. UDCA decreases the cytotoxicity of bile acids by decreasing the proportion of other bile acids and increasing the proportion of UDCA synthesised and secreted by hepatocytes, reducing the risk of liver failure.

Results of this research project demonstrated that administration of UDCA does not alter the bile acids tolerance test of normal healthy dogs, and it would appear that the bile acids tolerance test can be used to monitor hepatic function in dogs with liver disorders that are being medicated with UDCA.

- "Effect of oral ursodeoxycholic acid on bile acids tolerance tests in healthy dogs " Abraham LA, Charles JA, Holloway SA. *Aust Vet J*. 2004 Mar;82(3):157-60.

### **Improving therapeutic control of seizures in dogs**

(Investigator: Dr M. Govendir, Veterinary Science, University of Sydney)

This developed into major project with research conducted under three CRF grants during 2003-2008.

The first stage was a survey of veterinary practices that showed canine idiopathic epilepsy (IE) is the most common neurological disorder in dogs. Estimates of the incidence range from 2 to 3% of total hospital admissions. Dogs with IE generally remain on anticonvulsant medication throughout their lives.

The accepted therapy for minimizing the frequency and severity of seizures in dogs with IE was daily administration of phenobarbitone (PB) and/or potassium bromide (KBr). Most dogs treated with PB, KBr and GP either stopped seizing altogether or had a reduction in seizure frequency and particularly duration. These drugs are generally effective, affordable for lifetime treatment and allow dosing once or twice daily. However approximately 20-50% of epileptic dogs treated with PB alone will eventually demonstrate inadequate seizure control on PB even at high doses, and another therapeutic agent such as KBr must be added to the therapeutic regime to improve seizure control.

One of the aims of the study was to determine whether dogs with refractory IE would attain improved seizure control when gabapentin (GP) was added to the therapeutic regime. Research found that treatment with GP prevented PB withdrawal seizures and allowed PB to be completely withdrawn. Treatment with GP had no major side effects, but is more expensive. It was then important to investigate why long-term use of PB produced the side effects.

One of the side effects of prolonged PB medication is development of liver toxicity and the majority of patients medicated with PB for a long period had significant fasting hypertriglyceridaemia, but while not all dogs on anticonvulsant medication have high fasting fat levels, a small proportion do have significant fasting fat levels - not previously described in veterinary literature. Fasting triglyceride ranges for normal dogs had not been established and consequently this was done in this project. Fasting triglyceride and cholesterol concentrations may differ for various breeds of dogs.

The findings from research were disseminated in various reports;

- “Veterinarians’ anticonvulsant preferences for treating seizure disorders in dogs and cats”, E.K.Kluger, R. Malik and M.Govendir, Aust Vet J. (2009) 87(11):445-9.
- “Improving seizure control in dogs with refractory idiopathic epilepsy using gabapentin as an adjunctive therapy”, M.Govendir, M.Perkins & R.Malik, Aust Vet J. (2005) 83(10):602-8
- “Evaluation of triglyceride concentration in dogs using two portable meters”, E.K.Kluger, N.K.Dhand, R. Malik, W.J.Ilkin, D.Snow and M.Govendir, Am J Vet Res. (2010) 71(2):203-10
- “Serum triglyceride concentration in dogs with epilepsy treated with phenobarbital and bromide”, E.K.Kluger, R. Malik, W.J.Ilkin, D.Snow, D.R.Sullivan and M.Govendir, J Am Vet Med Assoc. (2008) 233(8):1270-7

### **Use of transcolonic portal scintigraphy to evaluate efficacy of cellophane banding for congenital portosystemic shunts in dogs**

(Investigator: Dr L. Abraham, University of Melbourne, Veterinary Science)

The objective of this project was to evaluate portal circulation in dogs following cellophane banding of single congenital intra- or extra- hepatic portosystemic shunts by use of transcolonic portal scintigraphy, and to determine if elevated post-operative cellophane- banded shunt fractions are due to failure of shunt closure (attenuation) or development of multiple acquired shunts using mesenteric portovenography.

The hypothesis was that in dogs with post-operative shunting identified following cellophane banding, the shunting of blood is due to development of multiple acquired shunts, rather than failure of complete attenuation (closure) of the shunt.

Research has shown cases of persistent shunting 10 weeks following cellophane banding of portocaval shunts by use of portal scintigraphy. The patients underwent a laparotomy and portovenogram and were identified with multiple portocaval shunts. This is an important development -the first world-wide follow up cases of persistent shunting with portovenography.

- "Use of transcolonic portal scintigraphy to evaluate efficacy of cellophane banding of congenital extrahepatic portosystemic shunts in 16 dogs " Landon BP, Abraham LA, Charles JA. Aust Vet J. (2008) 86(5):169-79

## 2007 GRANTS

### **Preliminary assessment of genetic markers of stenotic and regurgitant cardiac disease in dogs**

(Dr Mansfield, Murdoch University)

Cardiac disease is an extremely common problem in dogs and specific diseases have strong breed predispositions. Finding a genetic basis to such problems would be of great benefit. A possible genetic link has been found in humans between cardiovascular disease and an inherited systemic disorder of connective tissue, a condition known as PXE. The project is to determine whether this applies in dogs, and if so using the dog as the vehicle for further research. Method will be by comparison of affected and clear dogs by standard molecular genetic techniques of polymerase chain reaction and search for common polymorphisms.

Research findings were that the candidate gene did not correlate with cardiac disease in Boxers or Cavalier King Charles, but in Bull Terriers a homozygous mutation produced a high percentage of cardiac defects and this expression also correlated with survival.

Project concluded with partial success.

### **Evaluation of the hepatic microvasculature in dogs with congenital portosystemic shunts (CPS).**

(A/Prof. Hunt, University of Sydney)

Many canine liver problems are due to CPS, in which blood from the intestines largely bypasses the liver, and toxins build up in the system. Surgical treatment of CPS has a success rate of between 50% and 85%. In up to 50% of surgical cases the hepatic microvasculature are unable to develop sufficiently to accommodate a normal blood flow and acquired shunts develop in response to chronic portal hypertension. The study addresses this problem.

Twelve liver samples collected from dogs undergoing surgery for cellophane banding of portosystemic shunt were submitted for electron microscopy. Results showed obvious abnormalities interfering with transfer of various substances between the circulation and the liver cells. All dogs displayed serum biochemical evidence of hepatic dysfunction. Nine dogs had extrahepatic shunt and three dogs had intrahepatic shunt. Four main differences were determined in the livers of dogs with CPS compared to normal: defenestration, increased amounts of extracellular matrix, abundant micro- and macro-steatosis and narrow sinusoids. The endothelium seem to be thicker and the fenestrae replaced by large gaps caused by liver damage.

Future studies of the porous endothelium in CPS dog livers will enhance understanding of how the structural alterations affect the physiological bidirectional exchange between the sinusoidal vasculature and the liver parenchyma.

An article covering the research findings has been submitted for publication in a peer-reviewed journal.

### **Global gene expression profiling of canine lymphoma**

(Dr Nicholls, Murdoch University)

Cancer of the lymph system is a devastating problem in dogs and would form the most common tumour group of canine cancers seen clinically. Middle-aged to older dogs of both sexes are affected and it can occur in any breed, with a higher prevalence in some. It results in death 4-6 weeks after diagnosis if left untreated. This investigation has ramifications for both human and canine health and a possible sharing of information on these conditions. The hypothesis is that permanent alterations in gene expression underlie the pathogenesis of canine lymphoma and correlate with the clinical features/outcome of the disease.

The main costs are in purchase of Affymetrix chips which provide a micro array of some 46,000 genes, used to study altered gene expression in cancer cells in a sample of 30 canine tumour specimens obtained by biopsy.

The proposed outcomes of the research have been exceeded. Gene chip analysis has been completed on 3 lymphomas and 3 normal lymph nodes, versus the initial target of 2 and 2, and 49 lymphoma specimens collected rather than the target of 30. Some delays occurred due to relocation of the Gene Chip instrumentation to Royal Perth Hospital.

### **Molecular signalling mechanisms of eosinophilia in Rottweilers**

(Dr Mansfield, Murdoch University)

Eosinophilic disorders are a serious problem in dogs and Rottweilers in particular are predisposed to them due to exaggerated eosinophil responses to innocuous stimuli. The end result of excess eosinophils circulating is damage to organs such as the liver, lung and heart. Haematological survey of Rottweilers in Perth breeding kennels has shown a range of eosinophil counts and a familial predisposition for eosinophilia. Molecular biology technology is available to quantify the molecules responsible for eosinophilic stimulation which forms the basis of the project. If an association is found between cardiac disease and the identified genetic mutation the pattern of development of this disease will be more clearly defined, potentially leading to development of a genetic-based test to screen for affected animals at an early age.

The project is in the final stage. Samples from 24 dogs (6 non-Rottweiler controls, 6 Rottweilers with normal eosinophil counts, 8 Rottweilers with mild increases in eosinophils and 4 Rottweilers with very high eosinophil counts) were tested at Bristol University for measurement of the various cytokines that control eosinophil production, followed by assessment and preparation of a report for publishing in a peer-reviewed journal.

### **Use of the genome mapping technique for the rapid identification of MHC Class II haplotype in dogs with common autoimmune diseases**

(Investigator: A/Prof. S.Holloway, University of Melbourne Veterinary Science,)

The purpose of the research is to apply the GMT technology to the screening of large numbers of dogs with common autoimmune diseases such as immune mediated haemolytic anaemia, thrombocytopenia, diabetes mellitus, granulomatous meningoencephalitis,, Addison's disease and immune mediated polyarthritis. The project proposed to infer the MHC II type rapidly using a PCR process called GMT to amplify a polymorphic genetic element that is inherited with the MHC region in conserved stretches of the genome (conserved ancestral haplotype).

A DNA library has been created and there are approximately 500 samples from dogs with various immune mediated diseases from a variety of breeds. Common GMT profiles have been defined for most purebred dog breeds, which can now be compared with the profiles of diseased dogs in a rapid test. The GMT has been defined in identifying conserved haplotype in Greyhound dogs, and the utility of this shown in identification of duplicated haplotypes and in haplotypes that have deletions.

Research under the grant has been successful, providing the basis wide opportunities for further work.

- "Evaluation of the class II region of the major histocompatibility complex of the Greyhound with the genomic matching technique and sequence-based typing", published in *Tissue Antigens* (2008) 72:131-6.

### **Assessment of different methods for the treatment of canine aspergillosis**

(Dr Mansfield, Murdoch University)

Nasal aspergillosis is a relatively common problem in young to middle-aged dogs and has a high morbidity, usually resulting in euthanasia if left untreated. Treatment at present requires anaesthesia, is expensive and often a single treatment is not successful. The project aims to establish a method of CT imaging that will ensure effective drug distribution throughout the nasal cavity, and then compare results of various treatments with the drug clotrimazole against controls of the traditional clotrimazole soak.

The method of CT imaging has been established and is being used to determine drug distribution in clinical cases. Preliminary results suggest that currently used methods of treatment do not have as high a rate of success in Australian dogs as published reports would indicate.

Findings were presented at a national conference of Australian College of Veterinary Scientists and a paper submitted to a peer-reviewed journal.

- "Clotrimazole and enilconazole distribution within the frontal sinuses and nasal cavity of nine dogs with sinonasal aspergillosis" Sharman M, Lenard Z, Hosgood G, Mansfield C. *J Small Anim Pract.* 2012 Mar;53(3):161-7



### **Investigations into 'immunological memory' of dogs vaccinated with commercial vaccines against canine parvovirus and canine distemper in Australia.**

(Dr. Norris, University of Sydney)

This project was to provide important information on the duration of immunity in pet dogs following vaccination, and the usefulness of serology in immune memory. Ideally this would enable a more accurate and individualised approach to be made when designing CPV and CDV vaccination protocols for canine patients. It would also facilitate investigation of individuals with poor responses to vaccination and provide a powerful immunological tool with wide ranging implications on the study of canine infectious diseases. The project was to run over three years.

The project was delayed due to staff sickness and leave, and although progressing well, further loss of staff members vital to the project led the researchers to terminate the research and return all funding to the foundation.

### **The role of oxygen radicals in fatty liver diseases of dogs**

(Dr. Sandy, University of Melbourne)

The project aims to determine whether liver oxygen radicals are fundamental in driving the development of idiopathic fatty liver diseases in dogs. If they are, as expected, non-invasive diagnostic tests and development of a cost-effective treatment plan will be investigated. It is suspected that a fatty liver syndrome disease in dogs has a similar disease profile to the (nonalcoholic) syndrome in humans, and results also will provide some insights into the pathogenesis and possible treatment regimens in human medicine.

Final report delayed due to maternity leave and overseas study. Overdue.

## 2009 GRANTS

### **Magnetic resonance imaging (MRI) as a predictor of stifle pathology in naturally occurring cruciate ligament disease in dogs.**

(Dr. Andrew Dart, University of Sydney)

Cruciate ligament disease is the most common cause of lameness in dogs. This is a debilitating condition commonly affecting young adult large breed dogs and frequently affecting both stifles. Diagnosis is by physical examination, radiography and with visualisation at time of surgery via either arthrotomy (incision) or arthroscopy (endoscope). There is evidence that arthroscopy is inferior to arthrotomy in detecting cruciate ligament and meniscal injury and so it is generally preferred. However arthrotomy in healthy stifle joints leads to osteoarthritis. Arthroscopy is less invasive but still has inherent risks.

MRI imaging of the stifle joint for detection of cruciate ligament and meniscal injury is well established in humans. MRI of the normal dog stifle has been described but there is a conspicuous lack of research on the subject.

The aim of the project is to compare MRI to visual assessments of joints at surgery. The information gained from the proposed research will potentially be used to promote the noninvasive benefits of MRI as a diagnostic tool for stifle disease in dogs.

Final report has been requested.

## **Canine superficial pyoderma: should we be concerned about multidrug resistant Staphylococcus species?**

(Dr. Linda Vogelneust, University of Sydney)

Superficial bacterial pyoderma (pus-forming skin disease) is a very common secondary dermatosis in dogs. Historically in such cases, microbial culture has been considered unnecessary for diagnosis and treatment, as the causal bacteria are almost uniformly Staphylococcus species with a high and predictable antibiotic sensitivity. Recently, however, there is an apparent emergence of resistant Staphylococcus species around the world, similar to the trends with superficial pyoderma in humans.

Central to investigation of any infectious disease is determining which infectious agent or agents are responsible for the pathogenesis of disease. It is incorrect to assume that organisms resistant to antimicrobial agents have the necessary virulence factors to be involved in causation of disease. There is a need for information on ideal bacterial culture techniques. Skin surface bacterial culture may represent pathogenic bacteria or normal skin surface flora incidental to disease. It is also unclear if single or multiple bacterial species are typically involved.

The aims of the proposed study are: -

- To investigate the most appropriate method/s for bacterial culture of naturally occurring superficial bacterial pyoderma in dogs.
- To evaluate the species and antimicrobial sensitivity patterns for bacteria associated with superficial bacterial pyoderma in pet dogs, including the prevalence of methicillin-resistant or multi-drug resistant Staphylococcus species.
- Use specialised DNA hybridisation techniques to determine the location and likely involvement of each bacterial species within skin biopsies from pet dogs with naturally occurring superficial pyoderma.

Delayed employment of the dermatologist has held up progress. There has also been a reduced number of canine pyoderma cases, unsuitability of many cases due to localised lesions and difficulty with bacterial isolation technique. Concentration is on collecting more samples. The main bacteria causing pyoderma in dogs, Staphylococcus intermedius, has now been divided into additional species (*S. intermedius*, *S. pseudointermedius* and *S. delphini*) which are not easily distinguished with previously accepted techniques. PCR studies, requiring more expertise and expense are now necessary.

## **Characterisation of canine adipose-derived mesenchymal stem cells for treatment of diseases and disorders in dogs.**

(Dr. Paul Sheehy, University of Sydney)

Mesenchymal stem cells (MSCs) reside in various body tissues and play a role in cellular renewal processes in normal healthy animals. MSCs can be harvested and re-administered with the ability to differentiate into a range of tissue types for therapeutic purposes. However, the conditions required for high purity culture and unambiguous identification have yet to be determined.

MSCs have already been successfully used in humans for cartilage and bone repair, heart regeneration following cardiac infarction, neuronal regeneration and wound healing. Veterinary applications in dogs may include repair of fractures, cartilage repair, relief of osteoarthritis and treatments of hip dysplasia. While there is a large body of work investigating the basic biology of human MSCs and applications in various laboratory animal models, there is relatively little published research for the treatment of diseases of significance in the dog.

The specific aims of this project are;

- Establish cell culture methodologies from canine surgical waste (adipose tissue) based on published methods.
- Screen cell cultures for surface markers to optimise cell isolation procedures and population homogeneity.
- Evaluate cell labelling techniques for subsequent in vivo application.

Good progress has been made despite a hold up due to a staff problem:

- protocols and standard operating procedures for adipose derived stem cell isolation have been optimised
- protocols for collection of adipose tissue from clinical cases from within the University Veterinary Teaching Hospitals
- reagents have been sourced and purchased as required for tissue collection, tissue dispersion, cell isolation, propagation and cryopreservation
- now have a steady flow of samples
- obtained import permits for importation of commercial sources of canine bone marrow derived mesenchymal stem cells and culture media to be used as controls.

### **An integrated genomics source for the health and well-being of dogs in Australia.**

(A/Professor Peter Williamson, University of Sydney)

Many dog breeds are susceptible to specific diseases because of their genetic background. The dog genome has been entirely sequenced, and recent advances in DNA sequence data acquisition together with development of information technology provide a basis for a detailed dissection of the genetic component of these diseases, which can then be incorporated into disease management and eradication plans.

The fundamental biological resources, most commonly DNA (preferably with related material), that are the platform for such studies are most effective when organised into a well managed collection, commonly referred to as a “biobank”. The Faculty of Veterinary Science, University of Sydney is in a very strong position to be a leader in this area with its expertise in animal genomics and access to clinical cases of inherited diseases. An integrated Australian resource and collaboration with overseas veterinary schools will enable studies to determine genetic composition, determine disease gene prevalence, develop breeding and prevention strategies, understand disease pathophysiology, allow development of novel therapies and provide an educational resource.

Initial aims of the proposed project are:

- To collect samples from representative sires, dams and offspring of established dog breeds that are registered in Australia.
- To establish a curated collection of clinical case and control biological samples from dogs that enter the veterinary clinics of the university and associated veterinary practices as patients.
- To develop an integrated database with on-line links to existing public and veterinary resources.

The project has been launched, the biobank freezer is in place and collection strategies established. Curation is assigned and computer hardware supplied. Database structure with interface to records, database linkages established and tested, and sample collection commenced. Over 500 samples have been logged. A project to review archived and new pathology material has been commenced. Specific studies on the existing and ongoing sample collection have commenced, including:

- predisposition of genes involved in atopy
- inherited protein loss enteropathy/nephropathy in Soft Coated Wheaten Terriers
- gene expression as a method of detecting inherited disorders resulting from dysregulation
- molecular basis of Krabbe disease in Kelpies
- heritable sensory neuropathy in English Springer Spaniels
- susceptibility to mast cell tumours in dogs
- collection of samples from German Shepherds to complement studies (Dr. Bethany Wilson et al) on hip dysplasia, with other breeds to follow.

## 2010 GRANTS

### **Methods to reduce the severity and frequency of surgical infection.**

(Prof. Whittem, University of Melbourne)

Almost all dogs in Australia undergo elective castration or spaying. The cost of these surgical procedures is a considerable impost on pet ownership. Complications from this surgery due to surgical site infections (SSI) are usually unbudgeted additional pet-owner expense. As factors which predict the likelihood of SSI are identified, steps can be taken which will reduce postoperative wound infections.

This study aims to determine the incidence of and describe the factors associated with the incidence of SSI that occur in dogs undergoing surgery in a teaching and referral veterinary hospital in Australia. The study further aims to evaluate some specific strategies aimed to reduce the incidence and severity of SSI.

An interim assessment of progress revealed that the proportion of cases which had been enrolled which resulted in SSI was so low that the total number of cases available in three years of the project would be insufficient for any meaningful statistical analysis of the data. The project was therefore wound up. The infection control procedures in place at the university of Melbourne Veterinary Hospital for elective surgery result in a very low surgical site infection rate, which also precluded evaluation of treatments or interventions to reduce infection rate.

### **Loss of heterozygosity from chromosome 5 in canine oligodendrogliomas.**

(Dr. Long, University of Melbourne)

The spectrum of tumours seen in dogs is remarkably similar to that reported in people. Of the primary brain tumours seen, oligodendrogliomas comprise the third most common type. The mainstay of treatment in dogs currently comprises surgical debulking followed by irradiation. However, surgical removal of tumours is associated with up to 19% of dogs dying either during surgery or in the immediate post-operative period, and radiation therapy is currently limited in availability in Australia. The use of chemotherapeutic agents, either alone or in combination with other modalities would be beneficial in avoiding these problems. Given the loss of heterozygosity and sensitivity to chemotherapeutic agents in humans, the equivalent loss in canine oligodendrogliomas may also predict chemosensitivity, allowing these tumours to be treated by non-invasive chemotherapeutic protocols, thus improving survival for dogs suffering from these debilitating tumours.

Final report overdue.

### **Assessment of lidocaine as an adjunctive analgesic after abdominal surgery in dogs.**

(Dr. Haldane and Dr. Bryant , University of Melbourne)

If lidocaine is shown to have the same effects in dogs as it does in people and horses with regards to analgesic effects, anti-inflammatory activity and improved gastrointestinal function in the post-operative period, it will be of immense benefit to small animal veterinary practitioners, as lidocaine infusion is both inexpensive and easy to administer. This drug may reduce the requirement for high doses of opioids that can reduce intestinal motility as well as causing sedation. It may also reduce the requirement for anti-emetic or prokinetic agents postoperatively. There may even be an additional benefit in reducing time spent in hospital, both for dogs and for their owners in terms of quality of recovery and reduced expense for veterinary care.

Sick leave and a change of researcher, together with maternity leave have delayed this work. Resumption of research is scheduled for 2013.

### **Measurement of faecal cytokines in healthy and diarrhoeic dogs.**

(Dr. Mansfield, Murdoch University)

Cytokines are proteins produced in the face of inflammation, and are very important in mucosal surfaces such as the gastrointestinal tract. They are able to generate many other inflammatory mediators and are often the inciting factor for ongoing inflammation. Measurement of faecal cytokines may become an objective way to assess response to treatment and monitor individual animals. This would be preferable to invasive diagnostic testing such as endoscopy or surgical biopsies.

An early report shows progress has been good, with many of the bench-top issues solved. Healthy dogs have been assessed for the following faecal cytokines: IL-6, IL-8 and TNF-alpha. Assessment of IL-10, lactoferrin and IL-4 will be attempted next. Recruiting and testing of diarrhoeic dogs is progressing.

### **Finding glaucoma genes in the canine.**

(A/Prof. Baird, University of Melbourne)

Primary or inherited glaucoma is one of the leading causes of irreversible blindness in purebred dogs. Overall the prevalence of primary glaucoma is estimated to be nearly 1% across all dog breeds, but as high as 4-5% in the American Cocker Spaniel, Basset Hound and Chow Chow, and more than 1% across 19 other breeds. One of the major symptoms of canine glaucoma is an increase in intraocular pressure, which can rapidly cause blindness and is painful for the dog. Other symptoms include red, hazy eyes and dilated pupils.

The aim of this project is to use genes already associated with glaucoma in humans and assess their involvement in the canine species. By gaining direct evidence of involvement of these genes we can improve management of breeding practices and lead to eradication of the disease.

The project is under way, university ethics approval has been received and samples from dogs with glaucoma are being collected.

**Generation of ‘clinic ready’ canine induced pluripotent stem cells for regenerative medicine.**

(Dr P. Sheehy, University of Sydney Vet. Sci., \$30,000 total over 2 years)

The cells of animals have the potential to regenerate themselves, mainly in response to damage or disease. A contemporary therapeutic approach in veterinary medicine is to use this potential as a means to treat animals, and is termed regenerative medicine. This approach utilises cells with the most regenerative potential or “stem” cells, which proliferate and then turn into cells that have functional roles through a process termed differentiation. Adult stem cells that reside in specific tissues of adult animals have regenerative potential but typically the scope for differentiation is limited to the cell types within the tissue in which they reside. Embryonic stem cells (those harvested from embryos) have the potential to differentiate into any cell type (pluripotent), but require destruction of embryos to obtain them.

The aim of the project is to develop pluripotent stem cells derived from skin cells by reprogramming them. This project is a further development of the research group’s current CRF-funded project (2009-2010), entitled “Characterisation of canine adipose derived mesenchymal stem cells for treatment of disease and disorders in dogs”.

**Primary immunodeficiency in Australian German Shepherds.**

(A/Prof. P. Williamson, Uni. of Sydney Vet. Sci, \$32,000 total over 2 years)

Deficiencies of the immune system are a significant cause of infection with high rates of mortality in German Shepherd Dogs. Because of the complexity of the immune system, immunodeficiency may result from a wide range of underlying causes, and there is a strong heritable component. The most common form of immunodeficiency in GSDs is IgA deficiency, and the main aim of the project is to identify the underlying causes of IgA deficiency in Australian GSDs. IgA is an antibody important in immune response at mucosal surfaces. The experimental strategy is to obtain blood samples of representative GSDs and measure their molecular immune response of stimulated leukocytes and compare their profiles to identify deficient molecules or immune pathways.

**The use of multi-voxel proton spectroscopy to detect metabolic products in normal canine brains, and those affected by epilepsy disorders.**

(Dr S. Long, Melbourne University Vet. Sci., \$9880 total over 2 years)

The management of seizures associated with a variety of brain pathologies continues to be a problem in dogs, with a significant number of patients not responding to existing medication. In some forms of epilepsy in humans, magnetic resonance (MR) spectroscopy has shown increased levels of glutamate in some regions of the forebrain associated with the development of seizures, suggesting that drugs inhibiting the actions of glutamate may help in the management of seizures. The aim of this project is to use MR spectroscopy to identify whether similar changes exist in the brains of dogs with seizures, leading to the prospect of improved management of seizures.

### **NALP3 inflammasome activation in canine health and disease.**

(Dr. R. Sluyter, Wollongong University, Biol. Sci., \$10,000)

White blood cells are the main producers of the immune system hormones (IL-1 $\beta$  and IL-18) that are central in inflammatory and immune responses in humans, and have key roles in resistance to infectious disease. The aberrant expression and release of these inflammatory hormones is involved in the pathogenesis of many human autoimmune diseases, including rheumatoid arthritis, Type 1 diabetes and inflammatory skin disorders. Many human autoimmune and inflammatory disorders have equivalent disorders in dogs, yet very little is known of the action of inflammatory hormones in dogs.

The NALP3 inflammasome is a macromolecular complex involved in the pathway of production of these inflammatory hormones. The aim of the project is to improve knowledge about the activation of the NALP3 inflammasome to provide opportunities to understand the pathophysiology of various autoimmune and auto-inflammatory disorders and inflammatory skin disorders, as well as resistance to infectious diseases in dogs.

## 2012 GRANTS

### **Coxiella burnetii (Q fever): is this an important agent of disease in Australian dogs and reservoir for human infection?**

(Dr Katrina Bosward, University of Sydney)

The aims of the project are to:

- develop a canine specific serological test for detection of antibodies to *C. burnetii* and compare the relevant methodologies based on cost, ease and accuracy;
- determine the risk factors for dogs and humans.

Q fever is an important bacterial disease of animals and is transmittable to humans (zoonotic). It is considered one of the most common and serious of the zoonotic diseases. Given the 'flu-like' symptoms it is likely that the true impact in the community is underestimated. The role of *C. burnetii* in canine disease has not been clearly established. It is known that infected bitches have delivered dead puppies, with those surviving birth often dying soon after. Infection in humans can result in acute, chronic and subclinical disease, with a role in development of serious and occasionally fatal disease. Humans commonly acquire infection via inhalation of contaminated aerosols either directly from animals or indirectly via dust. A wide range of animals are considered important reservoirs for infection, the most frequently cited being production animals. While *C. burnetii* can be shed in large numbers in urine, faeces and milk, it is considered that the most potent are from birth products at parturition and milk of infected animals. A national vaccination program was instituted in Australia in 2001 and has successfully targeted workers in the meat and livestock industry; however recent studies have established that the relative importance of non-abattoir contact with livestock, wildlife or feral animals is increasing. There have been community outbreaks of Q fever in which dogs were established as the likely source of infection.

Confirming a diagnosis of Q fever is not straightforward. The researchers have already developed a serological test for feline *C. burnetti* infection in response to an outbreak in cats, and this line of approach is planned for infection in dogs.

**Naturally occurring bacteriocins; a novel therapy for the treatment of multi-drug-resistant *E.coli* urinary tract infections in dogs.**

(Dr Justine Gibson, University of Queensland)

Bacterial urinary tract infections (UTI) are common in dogs, and *E.coli* is the organism mostly frequently the cause. Many will be simple infections that are easily treated. Due to the rapid rise and spread of the multidrug-resistant (MDR) strains of *E. coli*, in both human and canine UTI, alternatives to conventional antimicrobial therapies are urgently needed. Bacteriocins provide an alternative solution. Bacteriocins are compounds produced by a variety of bacteria to inhibit the growth of closely related bacterial strains; they have a narrow killing spectrum and can be selected to target specific bacteria.

The aim of the project is to use bacteriological techniques to select bacteriocins that inhibit MDR *E. coli*, then identify the bacteriocin encoding gene. Once the target gene has been identified this can be cloned into a non-pathogenic *E. coli* for scaled-up production. Future research then will involve a mouse model investigating toxicity and half-life of the bacteriocin, and a bacteriocin protection study in mice with induced UTI prior to trials in a canine model.

This work has identified bacteria that inhibit the growth of multiple drug resistant bacteria that cause urinary tract infections. A grant was awarded for further work to commence in 2013.

**Characterisation of an antibody directed at Canine Telomerase Reverse Transcription.**

(Dr Sam Long, University of Melbourne)

Cancer represents one of the leading causes of death in older dogs. The spectrum of tumors that have been reported in dogs is remarkably similar to tumors seen in humans. With the development of treatments such as chemotherapy and radiotherapy, survival times for many tumors have steadily increased, however for some tumors survival is still only weeks or months. Increasing effort has been in developing therapies directed at novel targets, one of which is the enzyme telomerase. Telomeres are the non-coding regions at the ends of all mammalian chromosomes. Telomeres typically shorten with cell division, leading to aging and death of the cells. Telomerase acts to lengthen telomeres or prevent shortening, thus evading the pathways to aging and death of the cancer cells. Up to 90% of cancers in dogs so far examined contain telomerase. Telomerase has a component, telomerase reverse transcriptase (TERT), which catalyzes the lengthening of telomeres. Thus TERT has been proposed as a diagnostic and prognostic marker for a wide range of malignancies.

Only a few antibodies directed against telomerase have been developed and their specificities are under question. The aim of this project is to develop new antibodies specifically directed at detecting canine TERT to fully evaluate the presence of telomerase activity in canine tumors and to then develop telomerase-targeted therapies.



## **Structural and biochemical pathologies of canine epilepsy.**

(Dr Marjorie Milne, University of Melbourne)

Epilepsy is a common condition amongst dogs. Of canine patients presenting at veterinary surgeries with seizures, approximately 38% are 'symptomatic' and have an identifiable cause and 48% are 'idiopathic' and have no identifiable cause.

In humans, the neurotransmitter glutamate has been shown to be an important factor in the generation of seizures. Recent work has indicated that humans with epilepsy caused by brain tumours have abnormally high levels of glutamate and increased presence of specific proteins that transport glutamate. Other research has shown that glutamate is transiently elevated in patients with seizures due to idiopathic epilepsy. High levels of glutamate and glutamate transporters were identified in brains of four dogs with familial epilepsy, and were associated with abnormal electrical activity that triggers seizures.

The aim is to investigate whether glutamate levels or the expression of glutamate transporter proteins are increased in patients with seizures as a result of brain tumours. The first stage of the project has been funded by a university grant. This application is to fund the second stage, which involves the characterisation of glutamate activity and transport in patients with both idiopathic and symptomatic epilepsy. Proposed methods are:

- using high pressure liquid chromatography to evaluate glutamate levels in tumour tissue and the surrounding brain;
- using molecular genetic techniques to study expression of glutamate transporter protein genes in the same tissues.

## 2013 GRANTS

### **What is the genetic cause of haemangiosarcoma in dogs?**

(Dr Caroline O'Leary, University of Queensland)

Haemangiosarcoma is a cancer that arises from abnormal cells that line the inside of blood vessels. These tumours commonly occur in the spleen and heart. Clinical signs are usually not evident until the advanced stages of disease by which time the prognosis is poor. Although dogs of any breed are susceptible to haemangiosarcoma, this cancer occurs more commonly in certain breeds, suggesting a genetic basis for its development.

This project aims to use 'gene chips' to analyse DNA from dogs with a low risk of developing haemangiosarcoma and compare this against DNA from dogs in high risk breeds, with the disease. This comparison of DNA from the two groups aims to find DNA markers that are associated with increased or decreased risk of disease.

### **Development of a novel approach to evaluate immune responses in dogs**

(Dr Caroline Mansfield, University of Melbourne)

Currently to study immunity in dogs, they must be either housed in research colonies, or investigation is undertaken on naturally-incurring infection in client-owned dogs. Despite high ethical standards within animal facilities and measures of environmental enrichment, this still represents a significant impact on dogs. Prolonged time within research facilities can lead to behavioural issues and the rehoming of these dogs beyond the research project is often not possible. Disease investigations in client-owned dogs are not usually possible in early stages of disease, or in conditions that may occur relatively infrequently.

The use of alternative species based models would not eliminate the use of animals, but it would significantly reduce the logistical and ethical issues relating to the use of dogs. This project aims to develop a new model to study the immune system of dogs, using immune cells from dogs that are implanted into mice. Different types of donor tissue will be investigated to determine if this is possible, and if so, which donor tissue results in the best immune system development in the mouse. This model is then to be used to study the immune response to infection and vaccination without the need to conduct experiments on dogs.

### **The role of Kisspeptins in the management of ovulation in anoestrous bitches.**

(Dr Christopher Scott, Charles Sturt University)

Kisspeptins are naturally occurring proteins found within the hypothalamus (part of the brain). These have been shown to be pivotal to the brain control of reproduction in a range of species. In these species, intravenous infusion of kisspeptins have been used to initiate and synchronise the timing of the pre-ovulatory surge in luteinising hormone (LH) and hence ovulation.

This pilot study plans to investigate the potential of infusion with kisspeptin to stimulate ovulation in bitches. If a luteinising hormone (LH) surge can be predicted at a reasonably fixed time following kisspeptin administration, then this knowledge can be used in breeding management. Such treatment would also remove the need for repeated hormonal assays and vaginal cytology to determine optimal fertility, and thus, breeding times.

### **Assessment of deposition patterns of 99m Technetium labeled-Fluticasone propionate in the airways of healthy dogs using metered dose inhalers compared to nebulisation: a randomized cross-over study.**

(Dr Linda Abraham and Kathleen Chow, University of Melbourne)

Currently inhaled medications are in use for treatment of dogs with inflammatory airway diseases, despite a lack of research validating their use. This study aims to assess two devices in their effectiveness at delivering inhaled drugs into the airways and lungs of dogs. The devices are a pressurised metered dose inhaler (a 'puffer'), and a nebuliser. This will be achieved by labelling the inhaled drug with a radioactive tag, and imaging the drug after it has been inhaled using advanced imaging techniques to assess where inhaled substances are deposited in dogs (in the airways and lungs, or the device, spacer, face mask, mouth or throat).

This work will compare the puffer and nebuliser for delivery of inhaled drugs in dogs, to assess how effective each device is and whether one is better than the other, and determine if there is any relationship between the way the animal breathes, the number of breaths, and the amount of inhaled drug that is successfully delivered to the airways/lungs. The results will allow recommendations to be developed for the use of the puffers and nebulisers in dogs.

**Naturally occurring bacteriocins; a novel therapy for the treatment of multi-drug-resistant *E.coli* urinary tract infections in dogs.**

(Dr Justine Gibson, University of Queensland)

This project is a further development for a project that was granted funds for 2012. Work on that project has identified bacteria that inhibit the growth of multiple drug resistant bacteria that cause urinary tract infections. This work aims to characterise the proteins produced by these bacteria to progress the work towards commercial application. It is hoped these bacterial products will be of use in place of antibiotics.

## 2014 GRANTS

**Mapping canine sensorineural deafness in the Australian cattle dog**

(Dr Susan Sommerlad, University of Queensland)

Congenital hereditary sensorineural deafness (CHSD) is a significant problem in up to 99 different pedigree dog breeds. In Australia, it occurs at a prevalence of 11% in the Australian Cattle Dog. It causes deafness in puppies from about 6 weeks of age and impacts on their ability as working dogs, their behavior and their suitability as pets. Although the disease is widespread, we don't know the genetic basis of the disease and currently it can be reliably diagnosed only by Brainstem Auditory Evoked Response (BAER) testing. Knowing the genes involved would facilitate a DNA test that breeders could use to reduce the prevalence of the disease.

Our previous studies have identified a gene region involved in this type of deafness in the Australian Stumpy-tailed Cattle Dog. Here we will test this finding in the Australian Cattle Dog and use a more sensitive technique („gene chips“) to narrow in on the gene or genes involved. We will compare the DNA of bilaterally deaf dogs with DNA of normal hearing dogs to identify DNA markers that are associated with an increased or decreased risk of deafness.

The aim of this study is to undertake a genetic association study for congenital sensorineural deafness in the Australian Cattle Dog. We will:

- (i) identify bilaterally deaf and normal hearing dogs using BAER testing
- (ii) analyse their DNA using „gene chips“ that screen for 170,000 variants (“SNPs”)
- (iii) test for associations between the SNP variants and the risk of deafness.

### **What is the genetic cause of haemangiosarcoma in dogs?**

(Dr Caroline O'Leary, University of Queensland)

Haemangiosarcoma is a cancer that arises from abnormal cells that line the inside of blood vessels. These tumours commonly occur in the spleen and heart. Clinical signs are usually not evident until the advanced stages of disease by which time the prognosis is poor. Although dogs of any breed are susceptible to haemangiosarcoma, this cancer occurs more commonly in certain breeds, suggesting a genetic basis for its development.

This project aims to use 'gene chips' to analyse DNA from dogs with a low risk of developing haemangiosarcoma and compare this against DNA from dogs in high risk breeds, with the disease. This comparison of DNA from the two groups aims to find DNA markers that are associated with increased or decreased risk of disease.

### **Efficacy of a personalised tumour vaccine to treat dogs with cancer**

Dr Chris Weir, University of Sydney, Bill Walsh Cancer Research

The aim of this project is to test the effectiveness of our vaccine technology to treat dogs with cancer. A sample of the tumour from an individual will be collected and some of the proteins isolated. These are modified to increase the immune response to the cancer, aiding in the control of the disease. Unlike the more common vaccines that are used to prevent disease, this vaccine is used to treat disease. Similar approaches have shown benefit in both dog and human cancers. One group of dogs (n=10) will be treated by standard of care the other group (n=10) will receive standard of care plus a personalised vaccine made from its own tumour.

The overall aim of this project is to determine the efficacy of our autologous (personalised) vaccine technology in dogs with high grade cancer using cases with visceral haemangiosarcoma.

The specific aims are:

- 1) To identify dogs with visceral haemangiosarcoma;
- 2) To randomise dogs into either standard of care with placebo vaccine or standard of care and personalised vaccine and treat accordingly;
- 3) To monitor all dogs for signs of disease progression and compare data on the two treatment groups.

### **A comparison of hydroxyethyl starch 130/0.4, 4% succinylated gelatine and whole blood for the treatment of themorrhagic shock in dogs**

(Dr Lisa Smart, Murdoch University)

Shock is one of the most common problems encountered in dogs presenting to an emergency room; we see cases nearly every day at Murdoch University. Haemorrhagic shock, or shock caused by bleeding, is one of the most common types of shock we see in dogs. Haemorrhagic shock may occur with trauma, such as being hit by a car, or during surgery when there is excessive blood loss. Blood loss reduces the volume of circulating blood in the body and can lead to death if not treated promptly.

The treatment of haemorrhagic shock includes expanding the blood volume with fluid therapy so that the heart can pump blood properly again, and the organs can receive blood flow. Ideally, if a patient has bled, we would give them blood to restore blood volume. However, blood products are expensive, not readily available and may cause an allergic reaction if given rapidly. Therefore, fluids have been created that have artificial ‘proteins’ in them, called colloid molecules, which are supposed to mimic the effect of infusing blood. They fall into two main categories; starch-based colloids and gelatin-based colloids. Starch colloids last longer but recent research has shown them to have an adverse effect on blood clotting and kidney function, at higher doses. Gelatine-based products are excreted quickly from the body (within the hour) but may have less adverse effect on blood clotting and kidney function. They also appear to have beneficial effects on the body’s acid base balance, whereas most of the starch-based products can have an adverse effect on acid-base balance. However, little research has compared gelatin to starch-based colloids in dogs.

We have previously established that the most common starch-based product in Australia, Voluven, is effective at treating shock, when compared to standard fluid therapy. We also found that this does of Voluven had little effect on blood clotting (specifically, platelet function) as compared with standard therapy. However, we do not know if a gelatine-based product, Gelofusine, will perform the same or better in regards to the ability to resolve shock, acid base balance and blood clotting. If we can establish which product may be superior, we may be able to improve survival with the use fo the superior product, as we cannot only rapidly reverse the effects of shock but also avoid some of the detrimental side effects. These beneficial effects may allow us more time to deliver blood products ot the patient, if needed. This research is also applicable to the treatment of shock in people and this information can be used to help guide research in human emergency medicine especially military medicine where blood products in the field are limited.

Our objective is to compare the effect of 20ml/kg hydroxyethyl starch (HES 130/0.4), 4% succinated geelatine (GELO) and autologous fresh whole blood in dogs in haemorrhagic shock on cardiovascular parameters, acid base balance and platelet function.

### **Dogs, ticks and pathogens; the search for new and potentially zoonotic pathogens** (Dr Peter Irwin, Murdoch University)

Unlike the situation overseas, the diversity of tick-borne microorganisms carried by Australian ticks remains uninvestigated. The project will systematically characterise, for the first time, bacterial microorganisms in ticks infecting dogs in Australia, using next generation sequencing (NGS) techniques. In addition, ticks intercepted by Australian Quarantine authorities (Australian Biosecurity and AQIS) will be analysed in the same way to determine portental threats to the Australian dog population. The project is expected to provide important new information about potential pathogens transmitted to dogs by their ticks, the risks of introduced diseases, and will also shed light on the potential for the transmission of zoonotic pahtogens to humans by these ticks, particularly *Ixodes holocyclus*.

Aims:

1. To identify the species of ticks infecting dogs acorss urban and rural regions of Australia.
2. To systematically characterise the bacterial microorganisms within ticks removed from dogs in Australia (see aim 1) and compare this with the bacterial communitites with imported ticks.

3. To identify pathogenic and potentially zoonotic organisms in ticks feeding on Australian dogs.
4. To use the results (Aims 1-3) to improve our understanding about risks of tick-borne diseases in dogs and humans in Australia, and to guide further research.

**Investigation of cytokine concentrations in the cerebrospinal fluid of dogs with meningoencephalitis of unknown origin before and following therapy**

(Dr Susan Bennett, Murdoch.University)

Meningoencephalitis of Unknown Origin (MUO) is an inflammatory disorder of the brain and spinal cord that is common in dogs. The underlying cause of this disease is not known and currently there are no reliable markers to guide prognosis. The disease is associated with an aberrant immune response, with immune cells targeting and damaging the brain and other nervous tissue. The consequences of this are particularly severe given the very limited capacity of nerve cells to respond to damage. Treatment is based upon suppression of the immune system. However, despite treatment, the prognosis is generally poor with approximately 50% of dogs dying within the first month following diagnosis.

To date, there have been only a limited number of studies to assess the inciting and propagating factors involved in MUO. As a result, recommended treatment is based upon the results of small clinical trials and anecdotal evidence rather than targeting a specific type of immune response.

Cytokines are small molecules released from cells of the immune system that are integral in the coordination of the immune response. Studying cytokine profiles allows for the type of immune response to be further characterised and once known, selective immunosuppressive agents can be prescribed with optimal effect against the particular immune response.

Cytokine profiles have not been evaluated in dogs with MUO. Studying these profiles will increase our understanding of the disease mechanisms involved in the development of this life-threatening disorder. Assessment of changes in these profiles prior to and following therapy will help to elucidate the effect of currently recommended therapy, provide markers of potential prognostic significance, and aid the choice of additional immunosuppressive agents in dogs that fail to respond to conventional therapy.

Meningoencephalitis of Unknown Origin (MUO)	MUO is a diagnostic term reserved for the ante-mortem diagnosis of non-infectious, non-traumatic inflammatory central nervous system disease that does not meet the specific diagnostic criteria for specific
Inflammation (Inflammatory)	Inflammation is a general term used to describe the outcome of a response of the immune system to tissue invasion (by infections and/or foreign substances) or trauma (from external or internal insults).The
Immune response	An immune response is mounted by cells of the immune system and results in inflammation. These cells interact with each other to direct the type of immune response via many mechanisms, including
Prognosis	The chance of recovery from a disease.
Cytokine	Cytokines are tiny proteins released by cells of the immune system to initiate and mediate inflammatory responses. They may act locally or systemically and a specific milieu of different cytokines acting together

**Aim 1:** To report cytokine concentrations in the cerebrospinal fluid of dogs with meningoencephalitis of unknown origin before and following therapy. Specifically concentration of the cytokines INF- $\gamma$ , KC-like [CXCL-1], GM-CSF, IP-10, IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, MCP-1 and TNF- $\alpha$  will be measured.

**Aim 2:** To determine if individual cytokines are of value in predicting outcome and likely response to treatment.

**Aim 3:** In the subpopulation of dogs that fail to respond to conventional therapy, to assess cytokine concentration changes following addition of cyclosporine after an additional six-week period.

### **Guiding treatment regimes with anthelmintic sensitivity profiling in hookworms sourced from animal shelter dogs in Queensland/Australia**

(Prof Jacquie Rand, University of Queensland)

Diabetes occurs in about 1%-2% of dogs and current recommendations for management involve use of intermediate-acting insulin and glucose monitoring in hospital every 2-4 weeks in the stabilisation phase. This typically controls clinical signs but does not result in normal blood glucose concentration; blood glucose concentrations fluctuate considerably, with periods during the day above the level which causes glucose toxic damage to various tissues in the body, including the eyes. Currently, even with appropriate treatment, cataract formation occurs in 80% of dogs, frequently causing blindness and distress to the dog and owner, and is a cause of euthanasia. This project is designed to investigate improved methods of monitoring blood glucose concentrations and subsequent adjustment of insulin dose, aimed at achieving much improved control of blood glucose and prevention of cataracts. The new long-acting insulin detemir will also be trialled in the study, and a protocol for insulin dose adjustment developed. The availability of a new, compact continuous glucose monitoring system which can be used in the home, opens the opportunity for real gains in managing our diabetic patients.

This pilot study aims to compare traditional weekly in-hospital generated blood glucose curves and intermittent home blood glucose monitoring three times daily with continuous glucose monitoring over multiple days in the home environment in newly-diagnosed diabetic dogs, using a new compact continuous glucose monitoring system that provides data over 6-7 days (Medtronic i-Pro with Enlite-sensor). It is also aimed to develop an insulin dosing protocol in dogs for the new long-acting insulin, detemir. If blood glucose curves using continuous glucose monitoring provide superior information for dose adjustments in the initial stabilisation period to the more traditional methods, this project could be expanded to assess whether cataract formation is reduced in diabetic dogs as a result of superior blood glucose control using continuous glucose monitoring and detemir.

### **Development of an E. coli vaccine against canine pyometra**

(Dr Natali Krekeler, University of Melbourne)

Pyometra (pus in the uterus) is a life-threatening infectious disease in bitches. It is estimated that up to 25% percent of unspayed bitches develop the disease before ten years of age, and most of them die if left untreated. However, even if they are treated, most bitches will lose their reproductive potential, as a surgical intervention (spaying) is often the preferred treatment by veterinarians. *Escherichia coli* (*E.*

*coli*) are the bacteria most commonly isolated. So far no preventative measures other than spaying are available. *E. coli* carry many virulence factors, which facilitate their survival in the body. We propose to vaccinate bitches against these virulence factors and assess whether they develop an immune response. In a future study we would then infect the bitches with an *E. coli* strain that, we have shown, causes pyometra to determine if the immune response is protective against pyometra.

Aim 1 Determine if an antibody response can be induced by inoculating bitches with an *E. coli* strain that is expressing high levels of iron-capturing proteins

Aim 2 Determine which route, intramuscular or nasal, leads to a higher mucosal antibody response

Aim 3 Determine the type of antibody response induced

## 2015 GRANTS

### **Joint loading in agility dogs and possible association with chronic repetitive sprain injury of the carpus**

(Dr Winnie Shih, Murdoch University)

Sporting injuries in the racing greyhound has been an area of investigation in the recent decades but injuries to dogs performing in agility sport has not been in the focus of veterinary literature. Canine agility is a popular sport and there are currently more than 10 agility clubs across Australia with over 1000 dogs competing on a weekly basis. A recent studies have identified the A-frame and the bar jump as two of the top three most common equipment where injury occurs. A number of agility dogs presented to Murdoch University Veterinary Hospital have been identified with clinical signs of enthesiopathy of the accessory metacarpal ligament. These changes are consistent with a chronic repetitive sprain injury of the carpus. In a pilot study we have also documented extreme, non-physiologic hyperextension of the carpus in dogs as they enter the A-frame and completing the bar jump. Of additional concern, is the inequity of agility jump bar height across breeds relative to leg length. Currently the bar jump height is being determined on the height at the withers of competing dogs. There is concern that for some dogs, this may overload the carpus on landing. Based on our observations and findings in clinical cases, we suspect that repetitive loading during these activities with non-physiologic hyperextension of the carpal joint may cause chronic sprain of the accessory metacarpal ligament.



## **Evaluation of serial thromboelastography and platelet mapping in dogs with immunemediated haemolytic anemia treated with aspirin or clopidogrel.**

(Dr Christine Griebisch, University of Sydney)

Primary Immune mediated hemolytic anemia (pIMHA) is an immune-mediated disease which leads to premature destruction of red blood cells. It has a case fatality as high as 50-70%. The highest risk of death occurs during the first two weeks of therapy. pIMHA is associated with a hypercoagulable state and thromboembolic disease (forming of blood clots within the vessels). Venous thrombosis (within the veins) and pulmonary thromboembolism (within the lungs) (account for up to 80% of deaths in dogs with pIMHA). Thromboelastography (TEG) is a patient-side test that provides a global assessment of haemostatic potential and provides a graphical representation of clot formation over time. Studies performed using TEG so far have identified that dogs with pIMHA are hypercoagulable (increased risk of clotting) at presentation and that relative hypocoagulability (decreased clotting ability) at admission is a negative prognostic indicator. This relative hypocoagulable state has been proposed to result from disseminated intravascular coagulation (DIC) (a life threatening coagulation disorder), but this has not been confirmed. The use of anti-platelet drugs in dogs with pIMHA has been shown to improve short term survival but no study has used TEG to monitor response to antiplatelet therapy. Platelet mapping specifically determines the effect of specific antiplatelet agents on global coagulation by using different activators: arachidonic acid agonists to reflect the inhibiting effect of Aspirin and ADP agonists to reflect the inhibiting effect of Clopidogrel.

The aims of the current study are (i) To compare TEG, platelet mapping profiles and short term survival in dogs with pIMHA randomised to treatment with ultra low dose aspirin (ULDA) or clopidogrel, and (ii) To investigate using TEG whether DIC is the cause of relative hypocoagulability in dogs with pIMHA. Use of a patient-side test to detect hypercoagulability and development of an effective prophylactic strategy for hypercoagulability could markedly reduce fatality rates in dogs with pIMHA.

## **The role of gap junction channels, growth factors, and cytokines in the diseased canine cornea**

(Dr John Wright, University of Queensland)

This study will investigate (i) gap junction (GJ) (ii) growth factor, and (iii) cytokine expression after canine corneal injury and in ocular disease, with an emphasis on infiltrative diseases such as chronic superficial keratitis (CSK), pigmentary keratopathy (PK), and keratoconjunctivitis sicca (KCS).

To date, corneal repair in the dog has not been well-characterised and causes of corneal inflammation, cellular infiltration and pigmentation in the dog is poorly understood. Canine ocular injuries and diseases often present as emergencies and prompt, appropriate and sustained treatment are essential to maximise the repair process. Corneal damage is common in dogs due to trauma, detergent burns, and infections. Entropion, distichiae, corneal dystrophy, CSK, PK, and KCS lead to corneal dysfunction. Refractory corneal ulcers heal slowly; long-term administration of analgesia and antibiotics may be required, and sometimes surgery. Vascularisation, cellular infiltration and pigmentation often accompany the corneal repair process resulting in vision loss.

This project aims to provide knowledge on the disease processes affecting the canine cornea at the cellular level. The project will add significantly to the understanding of how the canine cornea reacts to trauma and inflammation and will result in the development of innovative treatments. Corneal healing is important, not only to re-establish the protective epithelium, but also for the restoration of corneal transparency and clear vision.

## **Development of Biomarkers for Canine Visceral Haemangiosarcoma**

(Dr Caroline O'Leary, University of Queensland)

Haemangiosarcoma is a lethal cancer that is common in some dog breeds including Golden Retrievers, German Shepherds, Labradors and Boxers. Diagnosis of visceral haemangiosarcoma (HSA) has not improved for decades, and the cancer is difficult to diagnose before it spreads. Potentially the first in the world, the research team has identified candidate biomarkers in the blood which can distinguish between dogs with HSA and HSA-like disease. This study aims to determine the accuracy of a newly developed diagnostic immunoassay for these biomarkers in 200 dogs with a variety of splenic diseases to allow determination of test sensitivity, specificity and accuracy.

**Assessing the global health of the colon: Understanding the changes in microbiome, short chain fatty acids, goblet cells and mucins in dogs with acute and chronic colitis.**

(Assoc. Prof Caroline Mansfield, University of Melbourne)

Colitis is inflammation of the large intestine, and is a common and uncomfortable disease in dogs that is manifested by straining to defaecate, with the stools often containing blood and mucus. Colitis often develops acutely at times of stress in dogs, especially when hospitalised. Dysbiosis (a bacterial derangement) in the colon may contribute to inflammation of the colon and development of clinical signs. Conversely, inflammation could in turn cause the dysbiosis. Regardless of the sequence of events sub-clinical colonic inflammation and dysbiosis may persist and take weeks to return to normal. In many dogs colitis is chronic and the cause is often classified as unknown or 'idiopathic', but may respond to some degree to fibre supplementation in the diet. This response is not always maintained, and diets with sufficient fibre may be unpalatable for dogs. Similar to the acute presentation, the exact cause and role of the colon microbiome in chronic colitis is unknown, but dysbiosis is also thought to play a role. This project looks at evaluating the bacterial diversity, bacterial function and mucin production in dogs with colitis as well as evaluating response to a novel prebiotic product.

## 2016 GRANTS

**Combination novel immunotherapeutics for the treatment of dogs with cancer**

(Dr Rachel Allavena, University of Queensland)

Cancer is an extremely common devastating disease in many breeds of pet dogs. The use of immune-based cancer treatments is of great potential in human and veterinary medicine. Intratumoural Complete Freund's Adjuvant (iCFA) injection and streptavidin-based autologous anti-cancer vaccine (Kvax) are two novel Australian invented, safe and effective, experimental immune-based treatments for naturally occurring cancer in pet dogs. To date we have tested these therapies individually against a range of very common naturally occurring cancers in dogs of many breeds, such as mast cell tumours, melanoma, and soft tissue sarcomas. We believe a combination of the local iCFA and body wide Kvax treatment will result in even higher cure rates and increased tumour shrinkage than each method alone. Individually, both treatments are safe and work against dog cancers in pilot studies in rodents and pet dogs conducted at our universities. Our aim is to assess the safety and effectiveness of single and combination iCFA and Kvax in pet dogs naturally suffering from a range of cancers that have been unable to be cured by currently available treatments. We will explore the immune mechanisms causing tumour shrinkage and cancers cures, and compare these with dogs who fail to respond to the treatments. By understanding how and why the treatments work; we will be able to optimise these treatments as new weapons against the cancers which commonly plague multiple dog breeds and bring heartache to countless dog owners.

### **Genetic variants associated with development of canine visceral haemangiosarcoma**

(Caroline O'Leary, University of Queensland )

Canine visceral haemangiosarcoma is a cancer affecting the internal organs, especially the spleen, liver and heart in many dog breeds, but especially German Shepherds, Golden Retrievers and Labradors. The cancer originates from cells lining the blood vessels and is not usually diagnosed prior to the cancer having spread internally in the body. Diagnosis and treatment of haemangiosarcoma has not improved for decades. However, better outcomes for dogs and their owners are likely if individual dogs at high risk of developing this cancer can be identified early using a genetic test, and then monitored for cancer development using ultrasound. Further, such genetic diagnostic tests could allow breeders to breed away from genetic markers which are associated with increased risk of developing this cancer. This study aims to develop such a genetic test by determining the DNA sequence from dogs with the cancer and comparing it to the DNA sequence from normal dogs to identify genetic changes which are associated with increased or decreased risk of developing the cancer haemangiosarcoma. Previous studies identifying genetic areas of interest in this cancer have been identified in by this group (some funded by the CRF), and the current study will take these findings further to identify actual DNA coding changes associated with the development and spread of this cancer. This will improve understanding about the development and spread of the cancer, helping to diagnose the cancer earlier and improve treatment.

### **Characterisation of pyometra-causing *Escherichia coli* in young and old dogs**

(Dr Natalie Krekeler, The University of Melbourne)

Pyometra is one of the most common, life-threatening reproductive diseases in female intact dogs. Pyometra not only affects the animal's health but also its breeding value. The pathogenesis of the disease is only incompletely understood. Previous studies undertaken at the University of Melbourne led us to hypothesize that the disease process differs between healthy young dogs and older dogs with uterine abnormalities. The aim of the present study is to characterize *Escherichia coli* (*E. coli*) isolates (bacteria that cause the disease) from young and old dogs affected by pyometra in order to investigate if bacterial strains between the two differ in their pathogenic profile. Another aim is to determine the presence of *E. coli* strains at different sites including the uterus, vagina and rectum and to type these strains in order to determine if the same strains are present at all sites. A third aim is to explore the biofilm forming potential of *E. coli* strains found in the canine urogenital tract. Bacterial biofilms have recently been the subject of investigation to explain recurrent disease, which is a feature of pyometra. In order to achieve these aims we propose to collect bacterial samples from dogs affected by pyometra and healthy control animals. The virulence of factor profile and biofilm forming potential for *E. coli* strains from young dogs will be compared with the profile of old dogs at the different anatomical sites. Healthy control animals of both age groups are included in the study.

### **Enteric Parasitism and the Aetiology of Diarrhoea in Puppies**

(Prof Richard Thompson, Murdoch University)

Diarrhoea in puppies is a significant and ongoing problem for breeders. Although there are many possible factors that can contribute to diarrhoea such as overcrowding, stress and poor nutrition, diarrhoea occurs in well-nourished and cared for puppies and it is widely believed that enteric parasitism is the cause. However, there is uncertainty about which parasites are responsible, how they are transmitted, and how best to treat and control infections. The aims of this project are to identify the factors that contribute to diarrhoea in puppies prior to weaning and to optimise approaches to treatment and control. We will investigate which intestinal parasites are most common in puppies, when they first appear after birth, and the intestinal bacterial flora. This will be achieved by regular, twice weekly, faecal sampling of naturally infected puppies from birth to weaning. Such an intensive study has not been undertaken previously. The information obtained will identify when anti-parasitic drugs (anti-coccidial and anti-giardial) should be administered in order to prevent parasite multiplication and associated diarrhoea. The project is innovative because we will simultaneously characterise both parasite and bacterial (the 'microbiome') communities in the intestine using sophisticated molecular, DNA sequencing approaches in puppies on the same diet but exposed to different treatment regimes, including the administration of probiotics.

### **Genetic management of canine lymphoma**

(Assoc Prof Peter Williamson, University of Sydney)

Cancer is a common cause of mortality in dogs. One of the most common forms of canine cancer is lymphoma, a cancer of the lymphocytes that accounts for approximately 25% of cancer cases. Lymphoma in dogs is in most cases treatable but not curable, with more aggressive forms leading to rapid decline and death within 1-6 months. The occurrence of this cancer is higher in some breeds compared to others, indicating that there may be specific gene mutations that cause the predisposition to developing the disease to be inherited. The aim of this study is to find the mutations that are causing lymphoma to occur and to use this information to inform dog breeders when deciding to mate their dogs.

## 2017 GRANTS

### **Distribution of genetic mutations associated with degenerative myelopathy**

(Dr Jayne McGhie, University of Queensland)

Canine degenerative myelopathy (DM) is a non-treatable disabling neurodegenerative disorder characterized by progressive motor neuron loss and paralysis that culminates in death (commonly by euthanasia). It affects mature dogs in a range of breeds. Recently a mutation in the canine superoxide dismutase 1 (SOD1) gene has been identified as strongly linked to the disease. Genetic testing offering a means to rapidly identify non-clinically affected dogs that are potentially at risk. This knowledge can be used to make breeding decisions to reduce the incidence of DM.

German Shepherd and related Shepherd breeds have a high incidence of DM. The current project aims to test non clinical working shepherd dogs and associated breeding shepherd populations (including any pups) to: (a) estimate the frequency of the deleterious mutation for DM, together with a selection of other known diagnostic markers of disease in these breeds, and (b) inform future breeding strategies to reduce the incidence of this disease in the population. In collaboration with the Queensland Police Force, we will use state-of-the-art genetic testing equipment (Sequenom) and expertise within the University of Queensland to genotype a random sample of 150-200 working dogs, breeders or pups. Expected outcomes beyond the aims above include development of a testing capability for disease diagnosis and preventative medicine that can be applied across a range of breeds in Australia, longitudinal study of the health and well-being of these working animals, and creation of a genomic biobank of DNA for German Shepherds for future research purposes.

### **Bacterial vaccine against canine pyometra**

(Dr Natalie Krekeler, University of Melbourne)

Pyometra (pus in the uterus) is a life-threatening infectious disease in bitches. Up to 25% of un-spayed bitches develop the disease before they reach ten years of age, and most of them die if they are left untreated. However, even if they are treated, most bitches will lose their reproductive potential, as a surgical intervention (spaying) is often the treatment of choice by veterinarians. *Escherichia coli* (*E. coli*) are the bacteria most commonly isolated. So far no preventative measures other than spaying are available. In a previous study we have tested a killed vaccine against certain bacterial virulence factors, which did not lead to the desired antibody response in the animals. This was most likely due to the fact that a killed vaccine was used. We now propose to generate a live-attenuated *E. coli* vaccine using a novel technique. Inactivating certain virulence genes in these bacterial strains has shown effective in a previous experiment with similar *E. coli* causing disease in birds. It allows using a live bacterial strain as a vaccine candidate, which is more likely to raise a satisfactory antibody response than a killed vaccine.

## **A safety trial of next generation cancer vaccines and targeted IL-15 plasmid therapy**

(Dr Christopher Weir, Kolling Institute)

Approximately 1 in 3 pet dogs will be diagnosed with cancer in their lifetime. While some canine cancers can be treated with surgery, chemotherapy and radiotherapy, expense and availability may prevent many dogs receiving treatment. Novel immune based therapies provide another potential weapon to fight canine cancer. We have developed a next generation cancer vaccine (NGV). The vaccine presents a patient's own tumour proteins back to the immune system together with a unique stimulant (RZ-1) and an adjuvant (Advax). Pre clinically we have shown this new vaccine approach is superior in efficacy to the currently marketed canine cancer vaccine. We have also developed targeted plasmid IL-15 therapy which is delivered directly into the tumour to stimulate immune cells inside. The next step is to show safety of the NGV with or without IL-15 therapy in canine cancer patients.

Canine patients with externally accessible tumours who have failed or have no option of standard of care therapy will be offered a place in the trial. Dogs will be placed into groups 1) Vaccine only 2) Combined Vaccine and Inter tumoural vaccine 3) IL-15 therapy 4) IL-15 + Vaccine

The dogs will be assessed for adverse reactions to the vaccine/IL 15 therapy during and after the treatment period and monitored for tumour progression or regression. Blood samples will be collected to assess immune cell levels and for cytokine analysis. Dogs showing no adverse reactions to the therapies and perceived benefit will be offered further treatments after a period of 3 to 6 months.

## **Biomarkers of canine glaucoma – early detection and disease progression**

(Dr Andrew White, University of Sydney)

Glaucoma is a leading cause of blindness worldwide with the incidence in humans similar to rates reported in dogs. The pathophysiology of glaucoma is complex, multifactorial and incompletely understood. Progress in our understanding of the disease, its management and the prevention of progressive vision loss, has therefore been slow.

The use of dogs over rodents as models of disease in ophthalmology has advantages including a larger eye that lends itself easily to surgical manipulation and a visual system that is adapted to vision in both day and night. In order to determine whether dogs can be used as an effective model of disease of glaucoma in human patients, we must determine how to monitor disease progression and/or response to treatment objectively and determine similarities and differences between the species. We will investigate several clinically relevant potential biomarkers in canine glaucoma. A biomarker is a measurable anatomic, physiologic, biochemical, or molecular parameter indicative of a normal or pathologic process, disease state, or of a response to an intervention. Biomarkers might include physiological measurements, blood tests, genetic, metabolic data, or measurements from images. We will look to objectively measure disease progression and determine if and when glaucoma and its progression can be detected earlier and more accurately than it currently is. This will subsequently allow development of clinical trials and interventions targeted at a much earlier stage, with potential for greater effects, compared to what is achieved with current treatments.

### **Imaging and monitoring osteosarcoma with a novel radiolabelled peptide**

(Assoc Prof Chiara Palmieri, University of Queensland)

Canine osteosarcoma is a common and aggressive bone cancer in dogs, with high mortality rate and less than 20% of affected patients surviving more than 2 years with standard therapy. The lack of specific and successful drugs and the prohibitive costs of treatment for the management of this tumour in dogs indicate the need for new diagnostic and therapeutic approaches. Cancer cells express many proteins that can be used as molecular targets. c-Met is a specific protein highly expressed in canine osteosarcoma and related to tumour growth and invasion. Developing a non-invasive and safe tool that could measure the expression levels of c-Met in real time would enable early diagnosis of canine osteosarcoma and facilitate the mechanisms to monitor the impact of treatment using new c-Met therapies. We will use a targeted diagnostic agent specific for c-Met to imaging and monitoring canine osteosarcoma in vivo. The key aims of this project will be the validation of this new imaging agent for osteosarcoma in dogs which will improve tumour monitoring (to identify residual cancer cells after therapy) and therapy selection (to identify patients who require further and/or different treatments).

## 2018 GRANTS

### **Towards eliminating canine hip-dysplasia: German Shepherd dogs as a model Distribution of genetic mutations associated with degenerative myelopathy**

(Professor Bill Ballard, University of New South Wales)

Canine hip dysplasia remains the most common heritable orthopedic condition in the dog world, This joint malformation (dysplasia) results in instability of the hip which ultimately causes damage to the articular cartilage and inflammation of the synovial membrane in the hip. Secondary osteoarthritis precipitates the clinical signs of lameness. The disease affects dogs of all breeds with different prevalence. Breed occurrence, as estimated by the Orthopedic Foundation for Animals (OFA), varies widely from 1 % to 75 %. A genetic model that can predict hip dysplasia has now been developed. This model could be our answer to minimising and possibly ridding the dog world of this albatross that is constantly thrown around our neck by the media and general public. However, it needs extensive testing.

We plan for three applications over a three-year period.

- In the first year of the project, our goal is to take 500 samples from German Shepherd dogs that have been radiographed and then see if we can confidently predict OFA results from their DNA.
- In the second year, we plan to extend the project to include 250 Labradors and 250 Golden Retrievers to see if we can confidently predict OFA results from their DNA.
- In the third year, we plan to develop breeding plans for these three breeds. This proposal will likely include less consumable costs but will include travel,

These three independent proposals will culminate in the first ever scientifically based breeding program to improve hip-scores in three common breeds. It will prevent the "throwing the baby out with the bathwater" mentality that has plagued many other DNA testing programs.

### **Assessing the canine uterine microbiome**

(Dr Natali Krekeler, The University of Melbourne)

Pyometra is one of the most common, life-threatening reproductive diseases in female intact dogs. The disease process is only incompletely understood. In recent years it has become obvious that bacteria can persist for a prolonged period of time in the bitch's uterus before causing an infection. We have shown last year that *Escherichia coli* (E. coli) bacteria from bitches affected by pyometra have the potential to form biofilm, enabling them to persist while evading the host's immune response. These bacteria are extremely difficult to diagnose because they often cannot be cultured with conventional methods. We hypothesize that the uterus of bitches is

not a sterile environment, but rather that bacteria often persist but cannot be readily detected. The aim of the present study is to characterize the "microbiome" (the combined genetic material of all present microorganisms) in the canine uterus with gene sequencing technologies. We propose to analyse samples from 10 young (s 4 years) and 10 old (£ 6 years) healthy bitches. They will be further split into two groups (n=5) with and without the uterine abnormalities commonly seen in pyometra. A second aim is to correlate the microbiomes with histological samples from these uteri. The results are aimed to lead to a better understanding of the disease process, which is a very important step in the development of new prevention strategies for canine pyometra and therefore, improved canine health.

### **The effect of diet on the host and microbiome metabolome**

(Assoc Professor Caroline Mansfield, The University of Melbourne)

The gut harbours millions of bacterial organisms, with the genetic potential of those organisms called the microbiome, which have an important role in health and disease. In particular, the impact of the gastrointestinal (GI) microbiome on the metabolic function of the host is immense. In people, the GI microbiome has been implicated in many metabolic diseases, including diabetes, obesity, depression and auto-immune disorders. To date, there are few studies looking at the metabolic impact of the microbiome in dogs. New analytical methods are now available to determine the metabolic output of the microbes present in the GI tract, along with assessing the hosts' metabolic status. In this study, we will evaluate the effect of diet change on the metabolome of healthy dogs and their microbiome. By establishing this, we can then extrapolate and develop further studies where specific manipulation of the GI microbiome can be initiated to combat specific metabolic conditions.

### **Genetic studies of canine glaucoma**

(Professor Paul Baird, The University of Melbourne)

Glaucoma is an eye condition that, if left untreated, can result in irreversible blindness. Glaucoma is one of the most common causes of irreversible blindness in purebred dogs. There is little understanding at present as to how glaucoma develops in dogs. If we can understand how genetics influences the disease, we can make a strong impact on how it is treated and managed in the future. Our aim is to undertake genetic studies in glaucoma affected canines to identify novel disease genes associated with canine glaucoma. We have already collected 150 samples from dogs (with and without glaucoma) and now we wish to use a "bead chip" to look at over 170,000 genetic variations scattered across all chromosomes in these samples. A total of 48 dogs across several breeds will be assessed to establish if the same or different glaucoma genes are present in each breed. Using statistical analysis we will be able to identify genetic variations that are only found in those dogs with glaucoma compared to those without glaucoma. As a result, new disease genes will be identified whereby we can improve the management and breeding practices in purebred dogs. Identification of novel genes through this research may also have profound implications in the study of human glaucoma as humans also develop glaucoma and a discovery from our research may help identify a new gene pathway of interest for further exploration in human subjects with glaucoma.

### **Canine lymphoma management by DNA analysis**

(Professor Peter Williamson, The University of Sydney)

Many canine cancers have a significant association with genetic background and are more prevalent in certain breeds. This project develops a strategy to manage lymphoma, a relatively common cancer in dogs, using DNA analysis. There are two aims, the first is to evaluate a DNA test developed to inform breeding decisions in Bullmastiff dogs. The second aim capitalises on our experience in working with lymphoma in Bullmastiffs, and

investigates the genetics of lymphoma in Border Collies. The expected outcome for the second aim is a DNA test, similar to that developed in the previous aim, that can be used by Border Collie breeders when deciding mating partners. Overall the goal is to manage cancer in dogs to minimise disease by preventative measures.

### **Genetics of mitral valve disease in Cavalier King Charles Spaniels**

(Assoc Professor Niek Beijerink, The University of Sydney)

**Background/Significance:** Myxomatous mitral valve disease (MMVD) is the most common acquired cardiorespiratory disease in small and medium sized dogs. It is also the most frequent cause of heart failure and heart disease related death. Severity of MMVD is highly variable with some dogs rapidly progressing into cardiomegaly and CHF, whilst others remain relatively asymptomatic throughout their lives. A progressive nature of MMVD is clinically recognized, however the underlying pathophysiological reasons are currently undefined or poorly understood. This represents a significant knowledge gap in disease pathogenesis. Heredity is suspected to play a major role in MMVD transmission. The natural history of this disease suggests prevalence, severity and mortality rates are higher in aged dogs, but there are many cases of severe early onset in certain breeds. The Cavalier King Charles Spaniel (CKCS) is utmost affected dog with prevalence approaching 100% by the age of 11.

**Aims:** By focusing on MMVD in one dog breed (CKCS), we aim to 1) perform a genome-wide association to identify candidate genes associated with the disease phenotypes; 2) perform comparative sequencing of positional candidate genes to identify potential causative mutations; 3) evaluate candidate mutations in an enlarged sample set including a variety of dog breeds. **Expected outcomes:** Significance and expected outcomes: The proposed study is of particular importance for canine health. This project has a realistic chance of revealing underlying mutations causing mitral valve disease in CKCS. Genetic tests to screen for risk of developing CHF could be made, giving dog breeders and vets the ultimate tools to make MVD a relative benign/asymptomatic disease in for future generations of dogs. Novel therapies for those dogs that would still suffer from CHF could be developed, possibly reversing this disease.

Assoc Prof Jan West

Trustee Canine Research Foundation

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