Mentor Abstract #1

Proposal for 2014 Summer Scholars Project

Project title: Design and Assessment of a Canine Pelvis and Limb Model to Teach the Diagnosis of Coxofemoral Laxity Secondary to Canine Hip Dysplasia

Principle Investigator: Mary Sarah Bergh DVM, MS, DACVS, DACVSMR
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Abstract:

Hip dysplasia is a common orthopedic condition in dogs that can lead to debilitating pain and lameness. Canine hip dysplasia (CHD) is characterized as a developmental disease resulting in excessive laxity within the hip, which results in secondary osteoarthritis. Diagnosis can be made on orthopedic examination with the Ortolani maneuver, or on radiographs. Early diagnosis of CHD can allow for treatment options that are not as effective in the adult animal or in later stages of the disease process. Data from one of our previous studies documented that 78% of surveyed recent graduates from the Iowa State University College of Veterinary Medicine were not comfortable in diagnosing canine hip dysplasia and 92% reported that they felt a customized pelvic limb (hip) model would help them learn or be more confident in performing the Ortolani maneuver. We hypothesize that we can create a three-dimensional model to effectively teach veterinary students how to perform the Ortolani maneuver and improve their comfort level and confidence in diagnosing hip laxity associated with CHD. The specific aim of this study is to create a canine pelvis and limb model to mimic a dog with coxofemoral laxity, and to test its efficacy as a learning tool for veterinary students. The Summer Scholar will gain an excellent working knowledge of the canine coxofemoral joint anatomy and hip dysplasia. With the Mentor's assistance, she/he will design and create the model as well as design and carryout a hypothesis-based research project to evaluate the effectiveness of the model. The end goal of the project is to write and submit a manuscript to the Journal of Veterinary Medical Education, with the Scholar's assistance. Funding to create the model will be provided by the Mentor.
Mentor Abstract #2

Project Title: Effect of glucuronides of vitamin D metabolites on expression of colon epithelial integrity genes in mouse models of colon cancer

Principle Investigator(s): Jesse Goff, Biomedical Sciences Dept. Iowa State University CVM jgoff@iastate.edu

Collaborating Investigator(s): Nicholas Koszewski, Biomedical Sciences Dept. Iowa State University CVM Nickkos1@iastate.edu

Abstract: We have an NIH R15 grant to determine the effectiveness of glucuronidated vitamin D metabolites on preventing colon cancer in mice. Epidemiological evidence suggests vitamin D insufficiency predisposes one to colon cancer. Other investigators have utilized the native hormone form of vitamin D=1,25(OH)2vitamin D to successfully treat mice but inevitably the dose that reduces adenoma development results in severe hypercalcemia. We discovered the glucuronidated 1,25(OH)2vitamin D (originally isolated from Argentinian plants) could deliver high doses of the hormone directly to the colon and could do this without significant hypercalcemia developing. We are utilizing 2 mouse models of colon cancer to test the therapeutic ability of these compounds. The mice for one model are already on the vitamin D treatments and will be terminated before summer begins. The other model will start shortly and mice will be terminated in early June. We hope the summer scholar can assist us with feeding and clinical assessment of the mice and necropsies of this second group. With luck the various indices of colon cancer (tumor burden, polyp size etc will be reduced in treated vs control mice as they spontaneously develop adenomas. We want the summer scholar to learn to do PCR to quantitate mRNA expression of genes involved in carcinogenesis such as β-catenins/WnT pathway genes and apoptotic genes. There are some standard biochemistry assays we expect to ask the student to learn and apply to frozen tissues too, such as a myeloperoxidase assay to assess inflammation in the tissues. Unfortunately a student cannot see the entire project thru from start to finish in ten weeks so we will get the animals started long before the semester ends and the student will work on one or two aspects of this project. Goff is planning studies out at the ISU dairy also (milk fever in cows and dehorning anesthesia in calves). The student is expected to help with all other projects our lab is doing – which generally serves as a chance to develop some clinical skills and get a break from lab work and get a basic and applied experience in a research lab.
Mentor Abstract #3

Project Title: The effect of lidocaine co-induction on cardiopulmonary parameters and propofol dosage in healthy dogs.

Principle Investigator(s): Bonnie L. Hay Kraus, DVM, DACVS, DACVAA
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Abstract:

Introduction: Propofol is an injectable anesthetic which provides smooth induction and rapid redistribution and elimination. Adverse effects include decreased systemic vascular resistance and myocardial depression leading to hypotension and respiratory depression/apnea. Lidocaine is a local anesthetic that is commonly used intravenously as a constant rate infusion for its anti-inflammatory and analgesic properties. Loading doses administered prior to induction quickly achieve plasma levels and decrease propofol dose. Adverse effects of lidocaine may also include hypotension and respiratory depression. The objective of this study is to assess the effect of lidocaine co-induction on propofol dose requirement and cardiopulmonary parameters in dogs.

Study Design: Prospective, randomized, blinded placebo controlled clinical trial.

Animals: Fifty client owned dogs (ASA I-II), presented for elective anesthesia.

Methods: Dogs will randomly receive saline or lidocaine 0.1 mL kg\(^{-1}\). Sedation will be scored twenty minutes after premedication with 0.1 mg/kg hydromorphone and 0.005 mg/kg acepromazine. Heart rate (HR), respiratory rate (RR), oscillometric blood pressure (SAP, MAP, DAP and pulse oximetry (SpO2) will be measured prior to premedication, administration of lidocaine or saline and propofol administration. Lidocaine or saline will be administered over 2 minutes. Propofol, 1 mg/kg, will be as administered IV over 15 seconds; additional propofol will be administered in 0.5 mg/kg increments until endotracheal intubation is possible. HR, RR, SAP, DAP, MAP, SpO2 and end-tidal carbon dioxide (EtCO2) will be measured at 0, 5, 10, 15, 20 and 30 minutes post-induction. Patients will be allowed to breathe spontaneously; presence of apnea (>30seconds without spontaneous breath) will be treated by manual ventilation at a rate of 4 breaths/minute, 15 cmH20 peak inspiratory pressure until return of spontaneous ventilation. Recorded data will include patient signalement, sedation score, propofol dosage, HR, RR, SAP, MAP, DAP, SpO2, EtCO2, presence of apnea, time to return of spontaneous ventilation and adverse reactions.
Mentor Abstract #4

Project Title: Development of a vaccine delivery device that will maintain life-long high titers of anti-GnRH antibodies.

Principle Investigator(s): Dr. Doug Jones
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Abstract:

Development of a single-dose non-surgical sterilant which is effective in both male and female cats and dogs would have an unprecedented impact on the global pet overpopulation problem. One approach to this problem is immunocontraception involving vaccination against gonadotropin releasing hormone (GnRH) which is a master reproductive hormone. The primary problem regarding this approach is that antibody levels are not maintained with traditional vaccine strategies. Thus far, research has focused on vaccine formulation (ie, adjuvants) while concepts of antigen delivery and persistence have been neglected. Our central hypothesis is that a vaccine strategy centered on persistent antigen delivery will result in the maintenance of high anti-GnRH antibody titers. The objective of this project is to design a vaccine platform consisting of a small implantable device. This implant will be designed to 1) release vaccine-containing nanoparticles over a long period of time and 2) release vaccine in response to low anti-GnRH antibody levels. The summer scholar will be involved in assessing the efficacy of the vaccine platform in a mouse model. The summer scholar will also be responsible for laboratory procedures such as ELISAs and Western blots in addition to in vitro experiments involving precipitation of antibody-antigen complexes in collagen-loaded implants.
Mentor Abstract #5

Project Title: Evaluating the role of regulatory T cells in canine immune thrombocytopenia (ITP)

Principle Investigator(s): Dana LeVine, DVM, DACVIM, PhD  
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Abstract:

Immune thrombocytopenia (ITP) is a common acquired bleeding disorder in dogs, causing frank and sometimes fatal hemorrhage. It is an autoimmune disease in which autoantibodies develop that target normal platelets for destruction. The underlying cause of ITP is unknown, so current treatments are too general, too often used, and have dangerous side-effects. Mortality rate of canine ITP is unacceptably high at up to 30%, meaning a novel treatment paradigm for ITP is direly needed.

In human ITP, regulatory T cells (Tregs) are thought to play a role in the loss of self-tolerance that leads to ITP. Human ITP patients have reduced T regs, whereas Tregs are restored in human ITP patients in remission (Bao 2010). Tregs have yet to be evaluated in dogs with ITP. Our project aims to define the role of Tregs in canine ITP.

**Hypothesis:** We hypothesize that Tregs are decreased in dogs with ITP and that Tregs provide a novel therapeutic target. The discovery of a T-cell regulatory defect will reveal new targets for novel immunomodulatory ITP therapies.

**Summer Aims:**

1. Establish a flow cytometry assay for canine Tregs based on previously established protocols (Mitchell 2012).

2. Determine whether Cocker spaniels, a breed highly predisposed to ITP, have lower Treg levels than dogs of other breeds. We will accomplish this by working with a local Cocker Spaniel breed group.

3. Determine whether dogs with thrombocytopenia of any cause have lower Tregs than dogs with normal platelet counts.

4. Begin the process of assessing Tregs in dogs with ITP compared to healthy controls and dogs with other causes of thrombocytopenia.

We may not be able to accrue enough ITP cases during one summer to achieve statistical significance for aim 4, however, the other aims will be achievable during the summer and will yield presentable and publishable results.
Mentor Abstract #6

Project Title: Evaluation of analgesics to treat chronic hoof pain in the horse.

Principle Investigator(s): Scott McClure, DVM, PhD, DACVS  
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Abstract: (300 words or less)

The objective of the project is to evaluate mechanisms to treat chronic hoof pain. This will be done using the force plate to measure Peak Vertical Force and Impulse in horses with induced lameness.

Lameness in the horse is the most common problem seen by veterinarians. Lameness is usually the result of pain. In the horse, there have been a number of ways to evaluate pain medications. Most evaluations measure the response to a noxious stimulus such as heat. This does not really provide a good evaluation of therapies for pain causing lameness in the horse. We have developed a reversible mechanism, using a band around the hoof, to induce a persistent lameness of 5 days duration. This model better mimics the receptors and responses seen in real life situations.

Clinically it appears that horses with chronic lameness tend to have decreased response to analgesics. This may be due to “ramp up” effects in the spinal cord or inflammatory cascades that are not invoked in short term studies. We will use this model to evaluate potential analgesics for persistent pain in the horse. We propose to induce a moderate lameness (3/5 American Association of Equine Practitioners scale). The lameness will be evaluated subjectively with a score from the AAEP scale from 0 to 5. It will also be evaluated using a force platform to determine how much weight is being placed on the foot. In a randomized crossover block design, the horse will receive an analgesic when 1 forelimb has the band tightened and when the band is tightened on the opposite forelimb, the horse will not receive an analgesic to serve as a control.

I am happy to discuss the project with potential summer scholars.
Project Title: Understanding the impact of the gastrointestinal microbiota on animal health through use of a gnotobiotic mouse model

Principle Investigator(s): Gregory Phillips, Ph.D.
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Collaborating Investigator(s): Michael J. Wannemuehler, Ph.D.

Abstract: Recent experimental evidence reveals that the bacterial communities (microbiota) that comprise the mammalian gastrointestinal (GI) tract can have a profound influence on the health of the host. Diseases ranging from colorectal malignancies to inflammatory bowel diseases (IBD) have been linked to an abnormal microbiota (dysbiosis) in humans and animal models. Despite the importance of bacteria to the wellbeing of humans and animals alike, how the microbiota influences health and disease is still not well understood. To better understand how specific microorganisms interact with the host, we are using a unique gnotobiotic mouse community, the Altered Schaedler Flora (ASF), which is comprised of animals colonized with only 8 known bacterial species. Despite the low complexity of the microbiota, ASF mouse exhibit normal immune system development and growth. Use of this resource includes monitoring the changes in relative number, spatial distribution and gene expression in response to alterations in diet and following infection with bacterial pathogens. Independent student projects include, but are not limited to, using PCR to measure changes in the abundance of individual ASF community members in response to infection with Escherichia coli, as well as identifying genetic changes in the ASF that occur in response to changes to the gastrointestinal (GI) tract. The ASF model also offers the potential to study exciting new results that indicate that the composition of the GI microbiota may actually influence animal behavior. The overall impact of these studies will lead to a better understanding of how the GI microbiota influences human and animal health and disease.
Mentor Abstract #8

Project Title: Digital Dermatitis Induction Model in Dairy Calves

Principle Investigator(s): Paul Plummer  
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Collaborating Investigator(s): Adam Krull, Jan Shearer, Pat Gorden

Abstract: (300 words or less)  
Digital dermatitis (DD) is an economically important polymicrobial disease process of dairy cattle that, despite 35 years of research, remains etiologically undefined. It is the leading cause of lameness in US dairy cattle. Although the disease is responsive to antibiotics, a definitive bacterial cause has not been identified. Treponema spp. are regularly isolated from PDD lesions, however attempts to induce classic disease lesions with pure culture of these microorganisms remain universally unsuccessful. Based on these findings, and the lack of efficacy of Treponema based vaccines, it is believed that the disease is polybacterial in nature. The identity of microorganisms that work in concert with Treponema spp. to cause the clinical presentation of DD in cattle remain unknown. Lack of this knowledge is an important problem because it prevents the development of effective intervention strategies that target the causative agents of DD. The overall objective of this application, and the next logical step towards the attainment of our long-term goal, is to develop a reproducible infection model that can be used to test hypotheses regarding the etiology and control of this disease. To achieve this objective we will be building upon and refining our current induction models utilizing naïve dairy calves in an attempt to consistently produce digital dermatitis lesions. The summer scholar student will give input on induction protocols, assist with inoculations, and monitor the progression of lesion development throughout the duration of the project. Upon successful completion of this research we expect to have a validated induction model for additional studies focused on digital dermatitis of dairy cattle. This model will be a critical tool in our ability to study and understand the microbial development of the lesions, as well as a means of testing vaccine targets for efficacy in a controlled environment.
Mentor Abstract #9

**Project Title:** Investigating Neurotoxic Mechanisms of Environmental Sulfide Gases

**Principle Investigator(s):** Wilson K Rumbeiha DVM, PhD, DABVT, DABT
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**Collaborating Investigator(s):** Steve Ensley, DVM, PhD, P Imerman Ph.D

**Abstract:**
The US population with neurodegenerative diseases is increasing and the environment is thought to play a causal role. These diseases are a significant burden to the patients themselves and to their caregivers. In the past 2 years our laboratory has made significant progress in developing mouse models of carbonyl and hydrogen sulfide-induced neurotoxicity. Carbonyl (COS) and hydrogen sulfide (H₂S) are environmental neurotoxic gases which may be contributing to the burden of neurodegenerative diseases. They co-exist in sewer gases, farm animal manure, and are both occupational hazards. Occupations associated with sulfide gas exposures include the petrochemical industry, livestock farming, manufacture of pesticides and other industrial products, and use of COS as a grain fumigant. The Department of Homeland Security has also designated H₂S as a potential bioterrorism weapon. In veterinary medicine, acute death of livestock from H₂S released by manure pit agitation causes significant acute livestock loses. Also, ingestion of high sulfur diets, such as renewable energy feed by-products, is a major cause of hydrogen sulfide-induced polio in ruminants. Although closely related structurally, the neurotoxicity of COS and H₂S has similarities and differences clinically and in neuropathology. Their neurotoxic mechanisms, diagnosis and treatment remain largely undefined or unknown. The summer research scholar will design and conduct rodent experiments to define neurochemical changes in the most critically affected brain regions using cutting edge technologies to define neurotoxic mechanisms of sulfide gases. The scholar will be exposed to career opportunities in neuroscience, hypothesis driven research, experimental design, scientific writing, and critical thinking skills in research. This research will advance our understanding of the potential contribution of neurotoxic sulfide gases to neurodegeneration and our knowledge of their toxic mechanisms. Ultimately, this knowledge will contribute towards mitigation, diagnosis and treatment of diseases caused by sulfide-induced neurotoxicity.
Mentor Abstract #10

Project Title: Evaluation of the efficacy of canine serum vs. plasma against protease activity for prevention and treatment of keratomalacia

Principle Investigator(s): Dr. Gil Ben-Shlomo, DVM, PhD
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Abstract:

Corneal ulcers are very common in dogs and with lack of appropriate treatment they present an imminent risk for eye and vision. If not identified early or if secondary infection occurs, degradation of corneal stroma (keratomalacia) caused by various proteases acting on corneal collagen may develop. This condition may lead to a rapid deepening of corneal ulcers and perforation of the eye. Serum and plasma are anti-proteases commonly utilized for prevention and treatment of keratomalacia. Despite their enormous importance and common use, the veterinary literature lacks adequate research-based information regarding the efficacy of canine serum vs. plasma against keratomalacia, over time and under different storage conditions. In addition, there is no information about contamination risk of serum and plasma over time when used for ocular treatment.

The goal of the proposed study is to compare the efficacy of canine serum and plasma against protease activity and their risk of contamination. Fresh serum and plasma will be collected from a healthy dog, determined by physical examination and complete blood work. The fresh blood will be separated into serum and plasma, which will be placed in a sterile dropper bottle as done in clinical setting and will be kept refrigerated at 4°C. A commercial kit will be used to evaluate the anti-protease activity of serum and plasma at different time points. At the same time points, the tip of the dropper bottles and their content will be cultured for bacterial and fungal growth to evaluate potential contamination. This evaluation will be also performed with serum and plasma kept at room temperature to evaluate the effect of storage temperature on efficacy and contamination risk. The results of this study will have an immediate and significant effect on the way serum and plasma are utilized for treatment of canine corneal ulcerative diseases.
Mentor Abstract #11

Project Title: Sequence analysis of the prion protein gene (PRNP) in the alpaca (Vicugna pacos)

Principle Investigator(s): Jodi Smith, DVM, PhD, Dipl. ACVP
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Jennifer Schleining, DVM, MS, Dipl. ACVS (VDPAM)

Collaborating Investigator(s): Justin Greenlee, DVM, PhD, Dipl. ACVP (USDA ARS NADC)

Abstract:
Transmissible spongiform encephalopathies (TSEs), or prion diseases, are a group of fatal neurodegenerative diseases affecting both animals and humans. Animal TSEs include scrapie in sheep and goats, chronic wasting disease (CWD) in cervids, and bovine spongiform encephalopathy in cattle. Although TSE has not been identified in alpacas, because of the potential for direct exposure to prion-infected animals or indirect exposure to contaminated environments, determining the potential susceptibility of alpacas to these diseases is important, especially considering the continued spread of the highly transmissible CWD agent in wild cervids across North America. Our long-term goal is to determine the potential susceptibility of alpacas to TSE agents circulating in the US. An essential first step in this process is analyzing the alpaca PRNP gene to 1) determine if it encodes prion protein with structural characteristics amenable to conversion to the disease associated form of the protein (PrPSc), 2) investigate sequence homology to other animals susceptible to TSEs, and 3) characterize PRNP sequence variation within the alpaca population. The rationale for the proposed research is that, in addition to providing the first characterization of the alpaca PRNP gene, once gene properties are known, logical hypothesis-driven research exploring the potential susceptibility of this species to TSEs can be performed. Thus, the proposed research will provide a necessary foundation for any future studies examining alpaca susceptibility to various TSEs, such as CWD. The project will include both field work (blood collection) and laboratory work (extracting DNA, amplifying and sequencing the PRNP gene, and analyzing sequence data). Preparation of a manuscript, with help from the investigators, for publication in a peer-reviewed journal will be encouraged. The potential exists for additional research experience on other ongoing TSE projects.
Mentor Abstract #12

Project Title: Understanding the development of epilepsy from rodent models

Principle Investigator(s): T. Thippeswamy, Professor
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Abstract: The main goal of the projects in my lab is to test whether preclinical neuroprotectants, when given soon after the symptomatic drugs following an acute exposure to a neurotoxin, for example kainic acid or organophosphates prevent delayed neurotoxicity and reduce mortality and morbidity. These neurotoxins induce seizures. Although seizures can be controlled in some individuals by using antiseizure drug such as diazepam, it does not protect seizure consequences at a later stage. The process of development of epilepsy, i.e., spontaneous recurrent seizures is called epileptogenesis. In spite of advancement in science and technology it not yet clear why some individuals become epileptic after a single seizure or following exposure to neurotoxin or traumatic brain injury. Several changes occur after first seizure and there is a phase called "latent period" with no obvious clinical signs of seizures after first seizure. We are interested in investigating the electrographic activity, neurobiological changes that occur in the brain during this latent period, in a way, “period of epileptogenesis”. We use both rat and mouse models. The projects involve, radio transmitter implant in mouse and rat for continuous EEG recording to understand real time changes in the brain during epileptogenesis. Brain, serum and cerebrospinal fluid (CSF) will be collected for various analyses at different time points after first set of seizures. The common techniques used are, histology and immunofluorescence, Western blot, EEG data analysis and behavioral testing for cognitive learning and memory. The summer project student will get the opportunity to work with postdoc and a graduate students to investigate cellular and molecular mechanisms of epileptogenesis.
Improving treatment of nematode infections using drug combinations.

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Parasitic nematodes infect a staggering number of humans and animals annually. In animals these parasites cause significant economic loss in food production and welfare problems in both companion and farm animals. In humans these organisms cause significant morbidity especially in children in developing countries. The large scale use of anthelmintic drugs has led to the development of resistance in many parasite species in a variety of hosts. Combined with other limitations, including a relatively narrow therapeutic window and less than 100% cure rates (especially in humans) there is a significant need for better and safer treatments. There is a growing body of evidence that while individual drugs are less than ideal, they may be much more effective as drug combinations than when used as single drugs. The project will address this hypothesis for parasitic nematodes. We will test this single drugs alone and in combination on individual parasite receptors (heterologously expressed in Xenopus oocytes) and also in whole nematodes to determine if effects are synergistic or otherwise. The project is part of ongoing research that involves techniques ranging from classical parasitology to molecular biology, receptor expression and electrophysiology.
Phenothiazine: Mechanism of action of an old anthelmintic

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Soil transmitted helminths, (STHs, nematode parasites) affect some 2000 million people and most domestic animals. Because sanitation and vaccines are not adequate for control, we rely on anthelmintics for treatment and prophylaxis. The continuous use of these drugs has produced resistance and the gives rise to an urgent need for new drugs to control these resistant parasites. This project is designed to investigate the mode of action of phenothiazine as an anthelmintic. It is an old but effective drug whose mode of action is not known. This project will test the hypothesis that phenothiazine acts as a cholinergic antagonist in nematode parasites, and acts in a similar manner to the novel drug derquantel. We will use larval migration assays and test the effect of phenothiazine electrophysiologically on nematode receptors expressed in Xenopus oocytes. The techniques are interesting and involve molecular biology, pharmacology, electrophysiology and parasitology. The project is part of a continuous study that is testing the modes of action of different anthelmintic drugs.