



TUFTS UNIVERSITY

CUMMINGS SCHOOL of VETERINARY MEDICINE

SUMMER RESEARCH TRAINING PROGRAM AWARDS 2015

ABSTRACT BOOK

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OIE Twinning Project

2015 Summer Research Proposals

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Awardee: Emily Dae Andersen V'17

Mentor: Dr. Felicia Nutter

Award Type: NIH

Research Project: Development of Sustainable Canine Gastrointestinal Parasite Control and Client Education Program in the Town of Samaná, Dominican Republic

Summary: Specific Aim 1: Determine the prevalence of gastrointestinal parasites in dogs in Samana, DR, with an emphasis on zoonotic taxa such as roundworms (*Toxocara canis*), hookworms (*Ancylostoma* and *Uncinaria* species), whipworms (*Trichuris sp*), tapeworms (*Dipylidium caninum*, *Echinococcus* species, *Taenia* species) and protozoa (*Giardia*, *Cryptosporidia*, *Entamoeba*).

SPECIFIC AIM 2: Develop effective gastrointestinal parasite educational handouts to distribute to owners of dogs taken to clinics run by Project Samana.

SPECIFIC AIM 3: Determine willingness to pay and develop feasible parasite treatment and control protocols based upon survey responses. Characterize the impact that educational materials have on the amount dog owners are willing to pay.

Methods: Fecal samples will be collected from dogs brought to Project Samana's clinic and will be analyzed for gastrointestinal parasites using basic microscopy to examine fecal smears and fecal flotations. Parasite prevalence and density will be reported. Dog owners' baseline knowledge of common canine gastrointestinal parasites and the diseases they cause will be assessed via verbal or written questionnaire. Education materials about these diseases will be created and distributed to dog owners in the form of printed handouts. A follow-up, same day written or verbal questionnaire will be conducted to assess initial knowledge gained and identical testing will be performed two weeks later to assess knowledge retention.

Significance: For over 20 years, Project Samana has conducted weeklong clinics twice a year in Samaná, DR with the goals of 1) providing necessary surgical and medical services to the animals of Samaná, and 2) educating animal owners in animal health care¹. This research will establish baseline prevalence of zoonotic gastrointestinal parasites in a subset of dogs that are brought to Project Samana to provide a basis for future project impact assessment. This project will also analyze the impact of educational handouts on owners' knowledge regarding these parasites, and owners' willingness to seek treatment for their dogs. Collected data will be used to refine education materials and to develop recommendations for canine GI parasite control and treatment protocols that are financially reasonable and acceptable to dog owners in Samana.

Awardee: Tiffany Baird V'17

Mentor: Dr. Flo Tseng

Award Type: USDA Formula Funds

Research Project: Epidemiologic Study of Wildlife Rehabilitation on the U.S. East Coast

Summary: Wildlife rehabilitation clinics provide a critical role in the community by accepting sick, injured, and orphaned wildlife from the public. The Tufts wildlife clinic has collected data on their wild animal patients since 1983, but it has never been examined in an aggregated format. The proposed study completes ongoing research that is currently being conducted on the 2004-2014 data and takes this research a step further by assessing needs across Massachusetts and establishing a more complete picture of wildlife rehabilitation across the US, with a focus on the East Coast.

The project has three main aims.

1. Use the 2004-2014 Tufts wildlife clinic data set to examine the strength of relationships between intake variables and final disposition.
2. Compare Tufts wildlife clinic data with other large clinics to develop a more complete picture of wildlife rehabilitation on the East Coast and across the U.S.
3. Assess the current status of wildlife rehabilitation in MA.

This study uses existing data sources (Tufts wildlife clinic, Massachusetts Division of Fisheries and Wildlife, an Atlanta-based wildlife clinic, and potentially other clinics across the U.S.) to describe the health of wildlife presenting to rehabilitation centers. SPSS will be used to conduct statistical analyses, including chi square tests, t-tests, correlation, and regression. In addition, GIS mapping will be used to better understand the geographical relationships between where wildlife are found and where they go to receive care.

This study will add critical information to the field regarding a variety of species that are regularly rehabilitated on the East Coast. In addition, the Tufts-specific analyses will help the wildlife clinic identify future research opportunities and strategically plan for the future.

Awardee: Arianne Baker V'17

Mentor: Dr. Claire Sharp

Award Type: NIH

Research Project: Prospective, Multicenter Evaluation of Transfusion Requirements in Dogs and Cats Following Trauma.

Summary: The overriding goal of the proposed study is to better understand the blood transfusion requirements of dogs and cats sustaining trauma. This understanding will serve several purposes:

1. It will better define the population and potential prognosis of small animal trauma patients with severe hemorrhage.
2. Provide data to guide blood banking practices in veterinary trauma centers.

We hypothesize that more severely injured dogs and cats, as defined by an Animal Trauma Triage score (ATTS) ≥ 5 are more likely to require a blood transfusion than dogs and cats with an ATTS < 5 . Additionally, we hypothesize that dogs and cats with an ATTS of ≥ 5 will be more likely to die or be euthanized than dogs or cats with an ATTS < 5 . Data for dogs and cats will be analyzed separately.

Awardee: Nicole Becich V'18

Mentor: Dr. Flo Tseng and Dr. Maureen Murray

Award Type: Private

Research Project: Health Survey and Hematology of Confiscated Psittacines in the Ecuadorian Andes

Summary: I will organize and execute the first general health survey of a sample of individuals of three commonly confiscated parrots of the genera *Amazona*, *Aratinga*, and *Pionus* from where they are held in quarantine at Bioparque Amaru Zoo in the southern Ecuadorian Andes. To date, no Ecuadorian zoo, rescue center, or veterinary hospital has undertaken a basic survey of the pathogens or parasites present in their respective population of confiscated wildlife. Since anywhere from 60-75% of newly emerging zoonotic diseases trace their origins to wildlife, and known zoonotic pathogens and parasites often spread through handlers' exposure to these animals blood and feces, establishing basic biosurveillance protocols for animals confiscated from unknown origins is a crucial step in developing biosecurity measures and data for reference in the event of a health crisis in either zoo animals or their human keepers. Additionally, it is important to determine if animals in quarantine that are possible candidates for re-release are carrying pathogens that may be dangerous to wild populations. This project will investigate the basic health status of a group of parrots commonly encountered in illicit trafficking seizures in Ecuador by means of physical exam, blood sampling for blood chemistry and parasites, establishing blood reference values for three species (*Aratinga erythrogenys*, *Aratinga Waglerii*, and *Pionus menstruus*), fecal sampling for estimated bacterial and parasitic load, necropsy protocol establishment for future execution by zoo staff, and tissue banking for future analyses. Collection of these data is important in establishing protocols for the intake and management of confiscated species, and setting an example for other zoos within the Ecuadorian Association of Zoos and Aquariums, for which Bioparque Amaru is the newly established headquarters.

Awardee: Alexandra Bemis V'18

Mentor: Dr. Chris Pirie

Award Type: NIH

Research Project: Ocular Coherence Tomography of the Optic Nerve in Canines

Summary: The purpose of this study is to assess the diagnostic capabilities of a portable spectral-domain ocular coherence tomography (SD-OCT) device which has been developed for human use in our veterinary patients. Since its inception, this technology has been cost-prohibitive for use in veterinary medicine, however, with continued technological advancements, these devices have become less expensive and could serve as a valuable diagnostic tool for veterinarians. There are few other modalities that can image the retina in vivo at the micron level. In physician based ophthalmology, SD-OCT can be used to aid in diagnosis of a variety of sight threatening conditions including vitreoretinal tractions, retinal detachments, retinoschisis, retinal edema, retinitis pigmentosa and glaucoma. It has been extremely valuable both clinically and in a research setting for its ability to obtain longitudinal measurements and monitoring disease progression. This study would be part of a greater body of work investigating whether this technology designed for humans can yield valid results in various animal species and establishing normative data. This study is novel in its specific focus on the optic disc region of the posterior segment in canines. The Optovue iVue SD-OCT scanner will be used on 10 healthy, adult Beagles to provide normative data for total retinal thickness, retinal nerve fiber layer thickness, disc area, cup area, rim area, and rim volume. Images will be collected from two operators to determine inter-rater reliability and each operator will take multiple images, repositioning the device in between so intra-rater reliability can be investigated as well. The automated values produced by the associated software using an algorithm designed for the human eye will be compared to manually adjusted values to test for clinically significant differences in order to determine the appropriate protocol.

Awardee: Rebecca Bishop V'17

Mentor: Dr. Thomas Jenei

Award Type: NIH

Research Project: Efficacy of Polymyxin B Therapy for Prevention of Endotoxemia and Laminitis in the Post-Surgical Colic Patient

Summary: Gastrointestinal disease continues to be of great concern to those in the equine industry, and is a common cause for presentation to ambulatory practitioners and referral centers alike. Colic is one of the most common diseases seen in horses presenting to equine referral centers, and despite many recent advances, post-surgical complications still present a significant threat to the long-term recovery of the surgical colic patient. Endotoxemia and laminitis contribute to significant morbidity and mortality in horses recovering from colic surgery; prevention of these costly and life-threatening conditions deserves great consideration.

Endotoxemia is defined as the presence of circulating endotoxin, which is released from gram-negative bacteria during cell multiplication or death. This triggers an inflammatory response through the release of endogenous inflammatory mediators, which manifests in the equine patient in numerous and varied clinical signs ranging from tachycardia and pyrexia to multiple organ failure, disseminated intravascular coagulation and cardiovascular collapse.

Polymyxin B, a lipopolysaccharide-binding antibiotic, has been proposed in many texts as a prophylactic measure to protect against the development of endotoxemia in horses recovering from colic surgery. At low doses, polymyxin binds the lipid A moiety of LPS on the bacteria as well as free circulating LPS, decreasing its half-life in the intravascular space and reducing associated inflammation. However, there is currently no literature directly demonstrating the efficacy of polymyxin for preventing development of clinical signs associated with endotoxemia or laminitis in colic cases.

Our aim is to examine the relationship between polymyxin therapy and the presence or absence of endotoxemia and laminitis in a retrospective, case-controlled study of horses that have undergone exploratory celiotomy for treatment of colic at the Tufts Hospital for Large Animals. If it is established that polymyxin prevents or reduces the severity of endotoxemia and/or laminitis in the clinical equine colic patient, it will be a great contribution to the practice of evidence-based medicine and will directly impact the ability of the clinicians at the Tufts Hospital for Large Animals to provide optimal care for their patients.

Awardee: Alyson Bradshaw V'18

Mentor: Dr. Sawkat Anwer

Award Type: OIE Twinning Project

Research Project: Evaluation of Antimicrobial Residues in Broiler Poultry and Fish in the Chittagong Metropolitan Area

Summary: The purpose of this study is to determine the identity and quantity of microbial residues in broiler chickens and fish being sold in the Chittagong Metropolitan City area in Chittagong, Bangladesh. The presence of antimicrobial residues in animal products that are sold for consumption can have potentially harmful effects to the human population, especially if the levels are above the upper threshold levels that are deemed acceptable for human consumption. Some of these deleterious effects include development of antibiotic resistant microorganisms, allergies, disruption of the gut microbial flora, obesity, and even cancer.

Previous studies have looked at the prevalence of antimicrobial residues in animal products, but there have been no formal studies done in Bangladesh. To assess the current status of these antimicrobial residues in Bangladesh, live birds and fish will be purchased from various outlets at five different

markets in the Chittagong Metropolitan City area. Questionnaires will be given to the outlet owners at the time of purchase to obtain information on the populations of birds and fish at the outlet, and the types of antibiotics used at the outlets. Some studies in other countries have shown seasonal variations in the levels of antimicrobial residues, so broilers and fish will be collected over the course of 6 months. Tissue samples will then be harvested and tested for antimicrobial products by both Thin Layer Chromatography and Ultra High Performance Liquid Chromatography. Additionally, an experiment will be done to determine if different processing techniques can reduce the levels of antimicrobial residues in the sample broilers and fish. Samples will be boiled, autoclaved, and cooked and subsequently compared to untreated samples.

This study will provide information on the quantity and identity of antimicrobial residues in the tissue of poultry and fish being sold in the Chittagong Metropolitan City area. Additionally, it will provide information about the possibility of reducing antimicrobial residue levels via different processing techniques. This information will be important for future endeavors looking to impact human health by reducing human exposure to antimicrobial residues.

Awardee: Isabel Francisco V'18

Mentor: Dr. Jennifer Steele

Award Type: Merit Scholars

Research Project: Knowledge, Attitudes and Practices Related to Schistosomiasis Transmission and Control in Leyte, Philippines

Summary: Schistosomiasis afflicts approximately 250 million people in over 70 countries [6]. In the Philippines, over 800,000 people are infected with *Schistosoma japonicum*, and another 6.7 million live in endemic areas [6]. These Filipinos are typically poor rice farmers with rudimentary water sources, sanitation and hygiene [6]. The national schistosomiasis control program focuses on surveillance and mass chemotherapy with the drug praziquantel as their main control strategy [6]. However, these efforts have been complicated by sporadic funding as well as low rates of compliance, and the disease continues to be a major public health concern. [7]

Studies at the Research Institute of Tropical Medicine (RITM) suggest that a multisectoral, integrated approach to schistosomiasis control might target the disease more effectively given its zoonotic nature [6]. However, the RITM also acknowledges a need for behavioral and sociocultural research to accompany any new policy formulation [7]. Disease awareness and concern remain low in rural communities, where both children and adults expose themselves to infected water in their daily practices [3]. Additionally, refusal to comply with public health programs such as annual mass drug administration (MDA) may be rooted in fear or misconceptions about praziquantel [3]. Results from this knowledge, attitudes and practices study could therefore inform plans for more effective disease control by addressing the sociocultural factors involved.

This study aims to assess knowledge and awareness of schistosomiasis in villagers of a local government unit or barangay in Leyte, Philippines. The study will also examine the effects of this knowledge on any preventive measures practiced by the villagers, and on their willingness to comply with public health control efforts such as MDA. A knowledge, attitudes and practices survey will be administered via interview to voluntary participants 13 years of age or older. I will be developing the questionnaire in collaboration with the RITM in order to assess (1) knowledge and attitudes related to schistosomiasis, its symptoms, and its transmission; (2) practice of preventive measures toward the disease; and (3) willingness to comply with public health control programs. I will then accompany community health workers who will administer the interview in Waray, the local dialect. I will actively analyze the data collected.

Awardee: Andrew Gestrich V'17

Mentor: Dr. Daniela Bedenice

Award Type: NIH

Research Project: Effect of Animal Age on Single-Dose Gentamicin Pharmacokinetics in Alpacas

Summary: Specific aims: The purpose of this study is to evaluate the effect of aging on aminoglycoside pharmacokinetics (PK) in a cohort of aged versus young-adult, healthy alpacas receiving a single intravenous dose of gentamicin.

Clinical Significance: Patient age can significantly impact the PK of parenteral therapeutic compounds, based on alterations in organ function (renal clearance, hepatic metabolism, cardiac output) and body composition (body water/adipose compartment) with increasing patient age. The latter affect not only the absorption and distribution, but also the metabolism and drug elimination, all of which determine the patient's drug exposure and thus dictate the appropriate antibiotic dose and dosing interval. Alpacas have an average life expectancy of 15-20 years, and have been imported and bred in the US in increasing numbers since the 1980s. This has, in turn, led to a rising population of aged animals presenting to the veterinary practitioner for medical care.

However, no previous research has examined the effects of advanced age on antimicrobial PK in this species. This paucity of pharmacological data currently hampers effective patient care and routinely causes practitioners to empirically treat camelids without due consideration of patient age.

Gentamicin is a predominant gram-negative spectrum antimicrobial which may target a wide array of intestinal, reproductive, urinary and some respiratory infections, for which alternate therapies are not readily available. However, the therapeutic window for gentamicin is narrow and little space exists between the borders of under-dosing the patient (thereby failing to adequately mitigate infection) and over-dosing the patient (thereby causing nephrotoxicity or ototoxicity). It remains essential to adapt dosing strategies for aged patients in order to meet therapeutic needs and counteract the development of antimicrobial resistance.

Experimental Design: The PK parameters of a single IV dose of gentamicin (6.6mg/kg) will be compared between 8 young adult (2-5yr), and 8 aged (>12yr) healthy alpacas, using a cross-over design. Blood samples will be obtained at designated time points following drug administration using an IV catheter, and frozen at -80°C until assayed by high-performance liquid chromatography. All procedures will be performed following written consent and IACUC approval. Pharmacokinetic fit and parameters will be assessed by specialized software.

Awardee: Kerry Gruendel Loughran V'17

Mentor: Dr. Andrew Hoffman

Award Type: NIH

Research Project: Urinary Exosomes and Cardiac Disease in Canines

Summary: Exosomes are nano-scale extracellular vesicles derived from the non-degradative arm of the endosomal pathway in cells. Specifically, multivesicular bodies contain lipid bound vesicles that are shed as exosomes into the extracellular spaces including body fluids (blood, CSF, urine). Exosomes contain nucleic acids (mRNA, miRNA) and proteins from the cytoplasm of their cell of origin. These biologically active molecules act as intercellular signaling systems, and due to shedding into biofluids reflect the health status of their parent tissues. Therefore, exosomes can provide information about cellular processes (cancer, repair, regeneration) occurring throughout the body and may contain important biomarkers useful for diagnosing and evaluating disease processes.

Urine is an underutilized reservoir for exosomal biomarker discovery that can be easily collected through noninvasive procedures and contains fewer contaminating proteins than other biofluids do. The first goal of this study is to optimize the isolation of exosomes from canine urine by comparing three different protocols, specifically i) ultracentrifugation ii) ultrafiltration coupled with size exclusion HPLC, and iii) precipitation. Ultracentrifugation is the most widely used exosome isolation technique currently available and does provide a reliable way to isolate exosomes from urine. However, ultracentrifugation is time-consuming, and requires equipment that is not always available in laboratory or clinical settings, and may result in a low exosome yield. We will measure the number of exosomes, quantity of exosomal protein, purity of protein, quantity of miRNA and purity of miRNA for each isolation technique in order to compare the usefulness of these techniques for downstream proteomic and genomic studies, including biomarker applications.

One of the main problems with treating congestive heart failure is drug-induced effects on renal function which induces a unique repertoire of exosomes in the urine, and complicates the use of urinary exosomes for biomarkers of cardiac disease. Hence we have designed an experiment to parse out the effects of cardiac versus renal (or both) disease on urinary exosomal biomarkers. Our study will evaluate candidate exosomal biomarkers in four groups of dogs (n=8 dogs/group), specifically 1) normal dogs, 2) dogs with azotemia due to renal disease without cardiac disease 3) dogs with congestive heart failure

without renal azotemia and 4) dogs with both cardiac disease and renal injury resulting in azotemia. We will quantify and analyze the exosomes, and exosomal proteins and miRNA in urine to look for distinct cardiac versus renal biomarkers. This study will be an important step towards making exosome analysis a useful and readily available tool for diagnosing cardiac disease, evaluating new therapies for cardiac disease and understanding the effects of cardiac treatments on the kidney in canine patients.

Awardee: Alexandria Hicks-Nelson V'17

Mentor: Dr. Benjamin Nephew

Award Type: NIH

Research Project: Behavioral Effects of Intranasal Oxytocin on Lactating Females Exposed to an Early Life Chronic Social Stress Model of Postpartum Depression and Anxiety

Summary: The aim of this project is to investigate the behavioral effects of intranasal oxytocin administration on lactating rat dams exposed to early life stress, given Dr. Nephew's previous research into a rodent model for postpartum depression and maternal anxiety. The chronic social stress (CSS) model is achieved by antagonizing F0 lactating rat dams with a foreign male for an hour a day for two weeks; the resulting maternal care in the F0 and F1 females can be characterized by sectioning off the cage with a low plastic grid to measure such ethogram entries as nursing, moving the nest, and standing away from offspring. CSS model F0 dams have shown depressed maternal care; depressed maternal care is displayed again in the F1 lactating dams that experienced the CSS model as pups of antagonized F0 dams. However, attempts have not been made to treat the CSS model at the level of the F1 dams, and thus, my project.

My hypothesis is that intranasal administration of oxytocin to F1 lactating dams that were raised by F0 CSS model dams will promote control-like maternal behavior in the F1 females. Oxytocin is a hormone known to play a key role in maternal bond formation and depression. Previous work done by the Nephew lab has shown altered oxytocin gene and receptor expression in CSS models and their offspring, raising the question of its role in the CSS model and transgenerational depressed maternal care. I will investigate the effects of oxytocin by following the CSS model in lactating Sprague-Dawley F0 rat dams, impregnating their F1 progeny, and administering intranasal oxytocin or saline to the F1 generation. Female F1 behavior towards their F2 progeny will be monitored by camera to be later reviewed for ethogram action tally and summation using ODLog. Data from the experimental groups will then be compared, along with a control group, using a one way ANOVA followed by Tukey's HSD for pairwise comparisons if there is an overall treatment effect. All graphs will be presented as a mean + SEM and the level of statistical significance will be set to $p \leq .05$.

Awardee: Michelle Lapointe V'18

Mentor: Dr. Elizabeth Byrnes

Award Type: NIH

Research Project: The Consequences of Pre- and Post-Natal Oxycodone Exposure: Examining Alterations in Maternal Care and Pup Vocalization Using a Rat Model

Summary: Extensive research efforts have examined prenatal exposure to substances such as cocaine, alcohol and nicotine and found significant changes in offspring growth, cognitive function, social behavior and vocalization (Sithisarn, Granger and Bada 2012; Behnke et al. 2013; Zeskind et al. 2014). However, little data has been collected on opiates, leading to gaps in the literature regarding both pre- and post-natal opiate exposure. Prescription opiate abuse by young and pregnant women has become a significant problem in the United States. In turn, increased research efforts are needed to determine the fetal risks associated with maternal opioid abuse (Davis et al. 2010; Johnson et al. 2011; Vassoler, Byrnes and Pierce 2014).

Employing a unique self-administration protocol, this project will lay the groundwork for understanding the effects of oxycodone abuse – both during and immediately following pregnancy – on offspring and maternal care behavior. Twenty-four nulliparous adult female Sprague-Dawley rats will be randomly divided into two treatment groups: oxycodone exposed (OC) and saline control (SC). Surgically implanted jugular catheters will allow OC females to self-administer oxycodone while SC females receive volume matched infusions of the vehicle. Drug exposure will begin three weeks prior to conception and continue through parturition. Six females in each group will then continue exposure while the remaining 12 females no longer receive infusions.

Maternal behavior will be assessed based on time spent grooming pups, latency to retrieve, and behavior in response to pup removal. The following parameters will be quantified by analyzing two weeks of video data. Pup weight will be recorded daily to examine early growth. Additionally, data on the number of ultrasonic vocalizations pups exhibit in response to repeated isolation will be used to examine changes in mother-infant communication.

It is hypothesized that opiates will decrease maternal care (Vassoler, Byrnes and Pierce 2014) and alter the pup's endogenous opioid system, changing the number of separation-induced ultrasonic vocalizations (Muller, Brunelli and Shair 2010).

Awardee: Eric Littman V'17

Mentor: Dr. Felicia Nutter

Award Type: Private Funding

Research Project: Radiographic Investigation of Normal Anatomy and Ossicle Density in Sea Stars in Comparison to those affected by Sea Star Wasting Disease (SSWD) or Ocean Acidification Conditions using Plain Film Radiography and Computed Tomography

Summary: Significance: Sea stars play a pivotal role as keystone predators of near-shore sub- and intertidal ecosystems along the western coast of North America. The large spatial expanse and high mortality rate associated with the current epizootic of Sea Star Wasting Disease (SSWD) will likely have resounding effects on biodiversity and ecosystem health. It is important to understand as much about this disease process as possible and potentially provide a means to diagnose the disease prior to the onset of external clinical signs.

Specific Aims:

Aim 1: To describe baseline radiographic anatomy and ossicle density on studied species of sea stars.

Aim 2: To determine if Sea Star Wasting Disease (SSWD)-affected sea stars have significantly reduced ossicle density as compared to clinically healthy sea stars of the same species *Hypothesis:* Ossicle density will decrease as the severity of SSWD increases.

Methods: At least two and up to five species of sea stars collected from Puget Sound will be examined by plain film radiography and computed tomography, and basic radiographic anatomy described. Ossicle density in clinically normal and SSWD-affected individuals will be assessed in relation to an established SSWD clinical disease scoring system.

Conclusion: This project will address an important knowledge gap by describing basic radiographic anatomy and ossicle density for multiple species of clinically normal sea stars. Based on this information, we will determine whether there are radiographic changes in sea stars affected by a devastating and poorly-understood epizootic disease, SSWD, which correlate with the severity of clinical signs. Finally, we will assess whether differences in basic sea water chemistry (calcium content) are associated with radiographic changes in the severity of SSWD. All components of this project will advance understanding of SSWD and the sea star species impacted.

Awardee: Rachel Madenjian V'17

Mentor: Dr. John Mandelman and Dr. Perry Bain

Award Type: NIH

Research Project: Evaluation of the Acute Physiologic Status of Cusk following Fishing Capture

Summary: Relatively little information is known regarding the bottom-dwelling teleost cusk, a National Marine Fisheries Service species of concern, whose population has been declining since the late 1960s. Presently, cusk are often taken and discarded as bycatch in hook fisheries targeting cod and haddock (1). As the stock populations for more coveted species like cod continue to diminish, cusk will become a more significant target in the Gulf of Maine recreational rod-and-reel fishery. In order to preserve the population and assess whether catch-and-release regulations are practical, the impact of recreational fishing on the species must be assessed, so practices can be enforced to promote survival of the population and cusk caught as bycatch.

Cusk suffer barotrauma and varying levels of injury when hauled up from the seabed. Although these injuries may appear life-threatening, there is evidence that cusk may survive if recompressed and released back to the ocean floor (2). We would like to perform blood analyses on captured cusk to determine the impact of capture and resultant injury on stress levels and to establish baseline hematological profiles for future work. The objectives of this study are to:

- Assess the acute stress response to a) capture and handling duration, and b) barotrauma and injury by measuring plasma cortisol levels and packed cell volume;
- Establish the baseline complete blood cell count and leukocyte differential count of cusk.

Understanding the physiologic stress faced by cusk following capture will provide insight about their ability to survive once returned to the seabed. Additionally, establishing baseline complete blood cell counts will aid in the assessment of delayed immune changes that occur as a result of stress in future studies that can manipulate stressors in a controlled environment.

Awardee: David McChesney V'18

Mentor: Dr. Andrew Hoffman

Award Type: NIH

Research Project: Experimental Observation of Canine Dendritic Cell Maturation Inhibition by Stem Cell Derived Extracellular Vesicles

Summary: Numerous inflammatory diseases burden the public health system. Examples include autoimmune diseases such as Multiple Sclerosis, Crohn's disease, and Rheumatoid Arthritis as well as

chronic inflammatory conditions such as osteoarthritis and chronic obstructive pulmonary disease (COPD). The vast majority of these diseases are incurable and many of them are poorly controlled.

Stem cell therapy offers a novel therapeutic to these conditions. However, there is a critical gap in knowledge concerning the mechanism by which these cells exert their effect. Mounting evidence supports that paracrine signals in the form of extracellular vesicles ('EV') derived from mesenchymal stem cells (MSCs) have an immunomodulatory effect on immune effector cells (T, B, NK, Macrophages, and DCs). The epigenetic signaling from the exosome carried in these EV's has been of particular interest.

In addition to understanding the mechanism by which stem cells produce benefits a realistic animal model is required before proceeding to human trials. Spontaneous diseases which afflict dogs are strikingly similar to human disease with respect to pathology, clinical symptoms, time-course, and patient management. Therefore canine derived cells will be used in this study.

The purpose of this research is to test the hypothesis that extracellular vesicles (EV) and in particular the smallest fraction of EV (exosomes, 30-100 nm) inhibit canine monocyte derived dendritic cell maturation, therefore providing an immunosuppressive effect. Concurrent studies are being performed on the T, B, NK, and macrophage immune effector cells in the Hoffman laboratory.

To test the effect of total MSC derived EV on the maturation of canine dendritic cells normal canine PBMOs (peripheral blood monocytes) will be incubated with one or the other EVs fractions (exosomes, 30-100nm, or microvesicles 100-500 nm), or control media and then exposed to T cell conditioned media which is known to promote maturation of canine CD14+ PBMOs to mature DCs. FACS analysis for the expression of MHC class II, CD1a, CD80, and CD86 molecules (mature canine DC markers) will then be measured to determine the resulting percentage of mature DCs in culture. TEM will also be used to further characterize the cells.

Should time allow, the results of the experiment may be further supplemented with gene expression analysis of the incubated PBMOs.

Awardee: Samantha Sangster V'18

Mentor: Dr. Hellen Amuguni

Award Type: USDA Formula Funds

Research Project: Analysis of Antibiotic Misuse in Livestock Management Systems in Kilosa, Tanzania

Summary: Antibiotic resistance is on the rise worldwide due to overuse in human medicine and livestock production. Resistant microbes compromise effective treatments in both human and veterinary medicine, especially in developing countries where there are greater financial and technological constraints on healthcare systems. Antibiotic resistance is a significant concern for

Tanzania from both a public health and economic standpoint. Livestock production is critical for many Tanzanians' livelihoods but the dual use of the same antibiotics in livestock production and human medicine and the multiple modes of transmission of resistant microbes from cattle to people compromises the efficacy of these drugs in human hospitals. Previous investigations by the USAID RESPOND project identified antibiotic misuse by cattle owners as a necessary area of research in the Kilosa district of Tanzania. Kilosa is an assemblage of villages practicing different livestock production systems including pastoralism, agro-pastoralism, peri-urban farming and is located in close proximity to Mikumi National Park. Antibiotic misuse by cattle owners in Kilosa can be attributed to the lack of drug knowledge, ease of acquisition of these drugs, and incorrect dosages and administration.

This project seeks to assess the antibiotic misuse in livestock production systems that may lead to an increase in antibiotic resistance of microbes to first-line, inexpensive antibiotics in the Kilosa district of Tanzania. We will perform antibiotic susceptibility testing on bacteria cultured from milk and fecal samples collected from cattle. Evaluation of antibiotic susceptibility will be based on CLSI standards classifying microbes as susceptible (S), intermediate (I), or resistant (R). We will use participatory methodology such as focus group discussions (FGDs) and key informant interviews in order to assess prevalence of disease, role of veterinarians and traditional medicine, and antibiotic usages at the community level. Conventional survey questionnaires for cattle owners will be utilized to determine what antimicrobials are frequently utilized, how these drugs are acquired, how they are administered in order to assess the extent of the misuse of antibiotics by individual cattle owners. Questionnaires will be analyzed with basic summary statistics. This study will be used to identify livestock management systems and communities within Kilosa where antibiotic misuse has resulted in the most resistance. USAID-One Health Workforce could then implement targeted education initiatives. Since similar livestock management practices are found across Tanzania these findings could be applied to other areas to help manage antibiotic use and educate cattle owners.

Awardee: Jordan Schachar V'17

Mentor: Dr. Randy Boudrieau

Award Type: NIH

Research Project: Treatment of Fractures of the Distal Radius and Ulna in Toy and Miniature Breed Dogs with Standard vs. Locking Plate Fixation: A Retrospective Study

Summary Fractures of the distal radius and ulna in toy and miniature breeds of dogs are difficult fractures to repair, and complications such as delayed or nonunions are not uncommon. It appears that plate fixation is one of the better fixation methods, as the degree of stability so imparted is critical for uncomplicated healing.

Standard plate fixation, however, can compromise the vascularity at the bone/plate interface, and this in bones that have a decreased vascular density of the intrasosseous blood supply at the distal diaphyseal-metaphyseal junction. Theoretically, improved vascularity can be obtained when using

locking plate fixation, as the blood supply at the bone/plate interface is spared; in addition, the fixed angle construct has some strength advantages in short bone fragments where there is limited space to place screws. These factors may improve bone healing and also reduce complications, e.g., reduce the infection rate. Whether these theoretical advantages can be substantiated in the clinical situation is the open question.

The objective of this study is to evaluate retrospectively the effectiveness of the standard Low Contact-Dynamic Compression Plate (LC-DCP®) and the Locking Compression plate (LCP®), either straight or T-plate, for the treatment of distal fractures of the radius and ulna in a series of toy or miniature breed dogs.

The medical records of adult (>6 months) toy or miniature breed dogs weighing < 6 kg, with fractures of the distal radius and ulna, will be reviewed. The inclusion criteria will include: fracture of the distal one-third of the radius and ulna and repaired with open reduction and internal fixation utilizing either an LC-DCP® or LCP® (straight or T-plate). Healing of these fracture will be evaluated based upon a grading criteria developed by the International Society Of Limb Salvage (ISOLS). All dogs will be followed radiographically until healing, and any short-term complications recorded. Long-term complications also will be assessed through telephone interview with the owners. A statistical analysis will be performed using a repeated measures ANOVA with post hoc Tukey HSD with comparison of healing between the two groups and the data obtained. Our null hypothesis is that there will be no difference in healing or complications between the different plating methods for these fractures: standard vs. locking plate fixation.

Awardee: Sarina Selleck V'17

Mentor: Dr. Sawkat Anwer

Award Type: OIE Twinning Project

Research Project: Prevalence of Rotavirus Infection in Cattle and Evaluation of Risk Factors for Intra and Inter Population Transmission in Southern Bangladesh

Summary: Rotavirus is an enteric virus that causes severe diarrhea in young animals and children. It is economically costly for livestock producers and its prevalence and transmission are not well understood in Bangladesh. In Bangladesh, there are concerns that the close proximity of livestock and people may lead to increased opportunities for transmission and reassortment of the virus between people and animals. Monitoring the current prevalence of the virus will establish a baseline comparison for future studies as well as the basis to formulate management and vaccination protocols.

The first aim of this study is to determine the prevalence of rotavirus infection in cattle in one geographic area of Chittagong, Bangladesh. To do this, districts will be randomly selected from the area

of study and herds within the district will be randomly selected as the study population. Fecal samples will be obtained from individual animals on selected farms and analyzed by ELISA at Chittagong Veterinary and Animal Sciences University. A representative number of positive samples will then be selected to be analyzed by polymerase chain reaction (PCR) to determine the strains of rotavirus present.

The second aim of this study is to evaluate suspected risk factors for intra and inter population transmission of rotavirus. To evaluate suspected risk factors, a questionnaire will be created to collect demographic and potential risk factor data about each animal. Risk factors will be compared with prevalence to determine if there are specific behaviors or management practices that are associated with a higher level of rotavirus infection.

This study will contribute to the growing body of knowledge about rotavirus in developing countries and will help aid producers in determining best management practices for minimizing the risk that rotavirus poses to cattle and humans.

Awardee: Giles Siddons V'18

Mentor: Dr. Patrick Skelly

Award Type: NIH

Research Project: Experimental Observation of Human Complement Inhibition by *Schistosomes*

Summary: Schistosomes are intravascular parasitic worms that infect millions of people and animals worldwide. Infection can lead to debilitating disease called schistosomiasis. There is no vaccine available to prevent infection.

Although schistosomes elicit an immune response while residing in the blood, killing of parasites does not appear to occur. Specifically, the complement system appears to be ineffective. Identifying the molecular mechanisms of complement evasion could lead to promising targets for novel drugs and vaccines. **The purpose of this research is to test the hypothesis that schistosomes impede activation of human complement, and to establish which proteins and activation pathways are affected.**

To observe which pathways of complement activation are affected by schistosomes, parasites will be incubated with normal human serum, and serum unexposed to schistosomes will be used as a control. Sera will then be tested for competency in activation of each of its three complement pathways using a commercially available assay.

Preliminary proteomic work from the Skelly laboratory shows that schistosomes cleave specific component proteins of the complement cascade (specifically C3 and MBL); concurrent with the above complement screen, the ability of schistosomes to cleave these specific proteins will be monitored.

Should time allow, the expression of schistosome surface proteins predicted to mediate complement disruption will be suppressed using RNA interference. Observation of subsequent changes in protein cleavage efficacy by suppressed versus control parasites should further elucidate the molecular mechanism behind complement evasion.

Awardee: Kenny Siu V'18

Mentor: Dr. Elizabeth Rozanski

Award Type: NIH

Research Project: Six Minute Walk Test; Establishment of a Reference Range in West Highland White Terriers and Validation of Home Measurement

Summary: The six minute walk test (6MWT) has been established in human medicine as a predictable method of assessing pulmonary capacity in people with lung disease, particularly pulmonary fibrosis. The 6MWT is performed by measuring the distance in meters an individual can comfortably walk in 6 minutes. The test is typically performed in a hospital or outpatient clinic, but may be performed at home using an IPHONE app. (<http://sixmwt.com>)

The 6MWT has been validated in dogs when performed in a clinic setting but has not been evaluated in dogs in at home. The first goal of this study is to validate the use of the 6MWT at home in dogs walked by their owners. This goal will be accomplished by recruiting 32 dogs from the Tufts Community. Dogs will be walked in the hospital by the student researcher and the 6MWT distance recorded. Dogs will also be walked at home by their owner using the IPHONE app. The order of walking will be randomized. The results obtained will be compared using a Bland-Altman plot to assess for degree of agreement between the two methods.

The second goal is to create a reference range for the 6MWT in west Highland white terriers, which are a breed of dog predisposed to the development of pulmonary fibrosis. Pulmonary fibrosis (PF), is a chronic progressive interstitial lung disease. Initial clinical signs include exercise intolerance and shortness of breath. Progression occurs over months to years, but the disease is ultimately fatal. Recent advances in human medicines may be applicable to dogs with early disease; however, currently disease in most dogs is detected at an advanced stage. We plan to use the IPHONE app to enroll ~ 500 WHWT around world to create a reference range in healthy WHWT with the hopes of being able to widely implement the 6MWT as a method of early detection of PF.

Awardee: Casey Smallwood V'18

Mentor: Dr. Hoque

Award Type: OIE Twinning Project

Research Project: HPAI Sero-Prevalence and Subtype Distribution in Commercial Farm versus Free-Range Ducks

Summary: Highly pathogenic avian influenza (HPAI) viruses were first detected in Bangladesh in March 2007 and since have become entrenched in the poultry industry. As a result, previous studies in Hong Kong, Thailand, and Vietnam have shown that disease introduction, spread and persistence are associated with the poultry industry as well as live bird markets. However, there are few studies in Bangladesh, specifically the Chittagong district, addressing these issues concerning avian influenza virus (AIV). Therefore, with a dense poultry population in Bangladesh and HPAI's (particularly H5, H7, and H9) appearing to be endemic in this population we are interested in investigating whether the domestic birds, specifically ducks serve as another reservoir host for AIVs. Using serological and oropharyngeal samples from both commercially farmed (caged) and free-ranging ducks, sero-prevalence of AIV will be estimated using ELISA and HI testing, and One-Step RT-PCR will be used for determining AIV subtype distribution. It is hypothesized that a significantly higher sero-prevalence will be seen in commercially farmed ducks compared to free-ranging ducks due to decreased living spaces, higher populations and therefore more interaction between birds in a commercial farm setting. Additionally, it is hypothesized that commercially farmed ducks will show significantly greater subtype diversity due to associated environmental factors (higher population, decreased space, etc.). Again these factors may increase interaction between birds, increasing disease spread and chance for strain mutation between birds. To analyze the results, I will perform descriptive analysis as well as a 2x2 Chi-square test to assess the association between factors (commercial, free-range) and binary results of AI serology presence (Yes/No). Additionally, we will use a univariate logistic regression to show the strength of association between the subtype diversity and environmental factors of commercial farm versus free-range (Odds ratio, SE, p-value and 95% confidence interval). Ultimately, the proposed study would demonstrate how proximity and population can affect disease prevalence, spread, and evolution. The future implications for this study might also increase not only awareness but also safety protocol standards for containment and preventive measures in terms of AIV outbreaks.

Awardee: Kelsey Turley V'17

Mentor: Dr. Claire Sharp

Award Type: NIH

Research Project: Evaluating the Complex Genetic Basis of Gastric Dilatation and Volvulus (GDV) in Purebred Dogs

Summary: Gastric dilatation and volvulus (GDV) is a common condition in large and giant breed dogs with an unacceptably high morbidity and 30-40% mortality rate. The German shepherd dog (GSD) is the most common breed of dog seen with GDV. Due to the importance of GDV, several large previous studies have investigated risk factors for the development of GDV. It is suspected that the pathogenesis of GDV is multifactorial, with both genetic and environmental risk factors contributing. We propose to further investigate the genetic component of this risk, as part of a larger study approaching GDV pathogenesis from a systems biology approach.

We began our investigations in the summer of 2014 by identifying 18 GDV affected and 148 control GSDs that have already been genotyped as part of their involvement in the Broad Institute DNA back. In the proposed study, our goal is to GDV phenotype other large and giant breed dogs that have been genotyped by the Broad, as well as genotype dogs presenting to the Tufts Foster Hospital for Small Animals with acute GDV. This multimodal approach to identifying cases and controls of at risk dog breeds will allow us to subsequently perform a genome wide association study (GWAS) of GDV in GSDs (discovered cohort) and other at risk breeds (validation cohorts).

We hypothesize that we will be able to explain a substantial proportion of GDV risk with single nucleotide polymorphisms identified in a GWAS. It is our hope that by identifying the predisposing genetic polymorphisms and their associated inheritance patterns we will ultimately be able to provide breeders with information to facilitate genetic testing and targeted breeding with the goal of markedly reducing GDV prevalence.

Awardee: Chelsea Van Thof V'17

Mentor: Dr. Flo Tseng

Award Type: Private

Research Project: Validation of Methodology for Detection of Fecal Cortisol Levels during the Rehabilitation of Golden-Cheeked Gibbons and Pygmy Loris.

Summary: Rehabilitation of wildlife can be both mentally and physically taxing to the animal, to the point where it can cause enough stress to suppress the immune system and prolong the healing process. Although there are multiple ways to collect glucocorticoid information, blood sampling may cause unnecessary stress and urine sampling can be unreliable in a group-housing situation. Fecal sampling is a non-invasive alternative used to collect measurements of stress hormone levels.

The aim of this study is to establish new protocols for measuring glucocorticoid levels in the Pygmy Loris and Golden-Cheeked Gibbon. Major glucocorticoid metabolites may vary immensely between the two species. Individuals of both species residing in the Dao Tien Endangered Primate Species Center, located inside Cat Tien National Park in Vietnam, will be involved.

Traditionally, HPLC has been used for glucocorticoid measurements, however, enzyme immunoassay (EIA) is a new method that is both faster and more cost-effective. Thus far, EIAs used on primates have targeted cortisol, corticosterone, and group-specific assays measuring reduced cortisol metabolites have been used. Glucocorticoid output varies from species to species and even between sexes of the same species, meaning that a specific EIA protocol must be established for every new primate species. HPLC must be used to validate these new protocols.

Sample collection will take place in Vietnam, while data analysis will be carried out at Pingtung University in Taiwan. Fecal collection will occur before and after naturally stressful situations, giving baseline and reactive glucocorticoid levels. Homogenized fecal samples will be used, ensuring equal distribution of steroid metabolites. Samples will be frozen after collection and during transport to Taiwan. Reverse-phase HPLC will be used to assess the pattern of metabolites measured and to characterize the specificity of the four GC assays tested. The highest detected EIA will be used in subsequent experiments.

Awardee: Gabriella Villanueva V'17

Mentor: Dr. Marieke Rosenbaum

Award Type: Morris

Research Project: Evaluating the Impact of a Free Spay-and-Neuter Program on Sterilization Rates and Attitudes Towards Sterilization of Cats and Dogs in Samaná, Dominican Republic

Summary: SIGNIFICANCE: Since 1993, Project Samana (PS) has sent veterinary teams twice a year to conduct a free spay/neuter clinic for the dogs, cats, and working equids of Samaná, DR. While PS subjectively believes their program is effective, project staff have been unable to demonstrate the impact PS has made on sterilization rates in the DR, nor have they sought structured feedback from the community. This research aims to fill the gap in knowledge that has been lacking for over two decades. Study results will provide quantitative information on the impact PS has had on sterilization rates in cats and dogs in Samana, and will serve as a baseline for future participatory impact assessments (PIA) of PS. In addition, results will aid in redirecting PS efforts to better meet the needs of the community and further its mission of enhancing animal welfare in Samaná, as well as serve as a model for the evaluation of other sterilization programs in Latin America.

SPECIFIC AIM 1: To assess whether there are higher rates of sterilization among dogs and cats in Samaná, where Project Samana has consistently operated over the last 22 years, compared to Nagua, a city where Project Samana has never operated.

SPECIFIC AIM 2: To identify the needs—with regards to surgical and medical animal care services—of adult men and women living in Samaná who own dogs and/or cats, and to compare these needs to the mission statement of and current services provided by Project Samana.

SPECIFIC AIM 3: To characterize the attitudes and perceptions of adult men and women living in Samaná towards Project Samana.

EXPERIMENTAL DESIGN: In **Part I**, the number of sterilized dogs and cats will be estimated and compared in Samaná and Nagua—a geographically distinct, yet comparable city where PS has never operated. Through random, systematic sampling, participants in both cities ($n \geq 100$ per city) will complete a survey composed of closed-ended questions focused on rates of pet ownership and sterilization, as well as knowledge, attitudes, and practices concerning sterilization. **Part II** will consist of a needs assessment to determine the overall attitudes towards PS and to determine the community-identified animal medical care needs through recorded semi-structured interviews based on PIA methodology.

Awardee: Emily Wheeler V'17

Mentor: Dr. Emily McCobb and Dr. Seana Dowling-Guyer

Award Type: Private

Research Project: Stressing for the Test: An Evaluation of the Relationship between Standardized Behavior Evaluations and Stress Behaviors in Shelter Dogs

Summary: Every year, an estimated three to four million cats and dogs are euthanized in animal shelters across the United States. At the same time, approximately four and half million people a year, mostly children, have reported dog bites, many of which required medical attention. Together, these two statistics highlight a challenge faced by animal shelters to try to balance the preservation of animal life with protecting the safety of the public; although ideally shelters would like to minimize the number of healthy dogs put to sleep, they also feel a responsibility to not put potentially dangerous dogs into the community. In order to address this matter, many organizations have turned to conducting behavior evaluations to determine whether or not an animal is suitable for rehoming. Concerns have been raised about the accuracy of the results of these evaluations. In recognition of this concern, many studies have attempted to evaluate and validate behavior evaluations. One such study looked at the behavior evaluation that is part of the Match-Up II Shelter Rehoming Program. It found preliminary data suggesting that standardized behavior evaluations could be inducing stress in dogs, consequently producing adulterated results. Therefore, the purpose of this study is to further investigate the effects of behavior evaluations on the stress levels of dogs by repeating a similar methodology with a larger and more diverse sample of dogs. Recordings will be made of a standardized behavior evaluation that is being conducted on dogs at two shelters in the New England area. Noldus Observer® XT Software will be used to code for and analyze stress behaviors observed during the evaluations, and these behaviors will be assessed for their correlation with stress. The insight gained from this study could provide a better understanding of the effect of behavior evaluations on dogs' stress and thus help assessors determine a more accurate score and make properly informed decisions about dogs' futures. The results of this study could also contribute to the validation of a formal rating tool that would use the presence or absence of behaviors to estimate stress level. This tool could be applied not only to shelter dogs, but also to dogs in a wide variety of stressful situations to promote improved welfare and superior decisions for dogs in the shelter and beyond.

Awardee: Elizabeth Wiley V'18

Mentor: Dr. Elizabeth Rozanski

Award Type: NIH

Research Project: Evaluation of Variations in Gastrohepatic Ligament Length in Dogs

Summary: Gastric dilatation-volvulus (GDV) is a common problem in large and giant breed dogs, as well as dogs with deeper chests. The underlying mechanism for the development of this condition remains unknown. The stomach is held in part in its anatomic location by the gastrohepatic ligament. One study documented that dogs with naturally developing GDV had longer ligaments than healthy dogs, but it was not clear if this was cause or effect. This proposed research study explores variation in gastrohepatic ligament length as a potential contributing factor to the risk of gastric dilatation volvulus in dogs with the hypothesis that longer ligaments would be a risk factor for increased stomach motility and subsequent volvulus. Dogs that are undergoing either abdominal exploration for any condition excluding GDV, or necropsy for which there is also an abdominal radiograph will be enrolled.

Thoracic conformation (the ratio of thoracic depth versus thoracic width), weight and body condition score (BCS) will be recorded. The abdominal radiograph is required to measure the 2nd lumbar vertebrae (L2) as an internal control. The gastrohepatic ligament will be measured using a ruler. The length of L2 and the ligament length will be recorded and the LL:L2 ratio be calculated.

Dogs with naturally occurring GDV will also be enrolled, to determine the ratio in affected dogs. In dogs without GDV, the ratio of LL:L2 will be correlated to thoracic conformation, and to body weight and BCS. This study would add to the knowledge of normal anatomic variables that could predispose a patient to GDV, and might serve as an interventional target if differences were identified related to thoracic confirmation.