TUFTS UNIVERSITY

CUMMINGS SCHOOL of VETERINARY MEDICINE

SUMMER RESEARCH TRAINING PROGRAM AWARDS 2013

ABSTRACT BOOK

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Merial Veterinary Scholars Program

Morris Animal Foundation

Nestle Purina Student Summer Fellowship

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Awardee: Travis Beeler V'16

Mentor: Dr. Alison Robbins and Dr. Donna Akiyoshi

Award Type: USDA Formula Funds

Research Project: *In vitro* Testing of Antifungal Agents Against *Geomyces destructans*, the Causative Agent of White Nose Syndrome in Bats.

Summary: White Nose Syndrome (WNS) is a fungal infection that is destroying hibernating bat populations across North America. Caused by the fungus *Geomyces destructans*, infection is associated with high mortality in exposed hibernacula. There is currently no treatment available for WNS; an effective environmental treatment may prove invaluable in containing the spread of infection and as an aid in raising healthy bat populations in captivity. In addition to environmental treatments, topical treatments could be used for the treatment of infected individuals. The aim of this study is to test the efficacy of various antifungal agents in inhibiting the growth of *Geomyces destructans*. This will be done by growing the fungus in cultures containing various antifungal drugs and comparing the growth between drugs. Drugs that prove successful in this study could prove to be candidates for *in vivo* testing.

Awardee: Kathleen Carey V'16

Mentor: Dr. Allen Rutberg

Award Type: NIH

Research Project: Effects of Porcine Zona Pellucida Immunocontraceptive Treatment on the Wild Horse Population of Jarita Mesa Wild Horse Territory, New Mexico

Summary: The federal government is required to maintain harmony between wildlife, livestock and other land uses without sacrificing ecological health. In order to do that, wild horse (*Equus caballus*) overpopulation must be managed. Porcine Zona Pellucida (PZP) vaccine is a widely used, effective and reversible wildlife immunocontraceptive. It has been used to treat an unstudied herd in the Jarita Mesa Wild Horse Territory (WHT), New Mexico. These mares were treated using entirely remotely delivered, single dose vaccine. Previously, the initial doses may have required boosters and were delivered by hand. Though designed for winter treatment, this was not possible with this herd. Therefore this herd provides a novel model for the efficacy of this vaccine as a method of population management.

The objectives of this study are to assess the effects of the vaccine on fertility as well as identify its possible influence on body condition, mare transfer rate and behavior in this herd. It will also be important to establish the baseline population of the Jarita Mesa WHT for the future assessment of long-term population level effect.

My role in this study will be to observe the wild horse herds of the Jarita Mesa WHT to collect a variety of data. I will determine fertility rates of treated and untreated mares, identify as many horses as possible and add new horses to the Wild Horse Management Identification Management System for future reference. I will obtain weekly body condition data, identify mare subpopulation transfers and observe sexual behaviors as parameters for herd health in relation to treatment and as a foundation for future study. I will analyze these data statistically to determine if fertility is related to date of treatment and identify the success of the treatment methods employed to guide future treatment.

With further knowledge of the effects of this treatment, its applicability and use may increase. As a wild horse population management tool, it could reduce population to within federally determined optimum levels and ease tension among officials and ranchers. It would save horses, land and economy from the harm of overgrazing and it would address the issue of the thousands of horses that have been removed from rangeland without being adopted out. Unsustainable long term holding facilities supported by taxpayer dollars would not have to account for the lack of demand for range horses in the national market.

Awardee: Kelly Chevett V'15

Mentor: Dr. Sandra Ayres

Award Type: NIH

Research Project: Efficacy of a Short Progesterone-Priming Protocol on Pregnancy Rates in Anestrous Does During the Non-Breeding and Transitional Breeding Season

Summary: Goats are seasonal breeders, and it would be advantageous to farmers to breed their stock multiple times a year. Inducing goats to cycle has been done with some amount of success, but pregnancy rates are often low on the first breeding as the ovulation is usually infertile. In order to induce cycling, hormonal control must be manipulated. The specific aim of the project is to compare pregnancy rates in goats, bred on either the first or the second induced estrus of the non-breeding season. Twelve female Alpine and Saanen dairy goats will be divided into 2 groups. All animals will be induced to cycle using a short progesterone priming protocol. Group A will be bred on this first induced heat. The remaining animals will not be bred, but allowed to continue this first estrus cycle for 7 days. At this time the animals will be short-cycled using PGF2α, and bred on the resulting second estrus. Blood sample will be collected and analyzed to measure progesterone levels. This will be done daily during synchronization and breeding, and then two to three times a week during early pregnancy.

Awardee: Shailey DeVito V'15

Mentor: Dr. Elizabeth Rozanski

Award Type: Purina

Research Project: 25-Hydroxyvitamin D Levels and Development of Respiratory Infections in Puppies

Summary: Puppies frequently develop tracheobronchitis and subsequent pneumonia. This can lead to complications such as systemic inflammation and respiratory distress that require extensive treatment, and may even result in death.

Puppies that enter the shelter are at increased risk of developing tracheobronchitis and pneumonia due to exposure to other dogs, stress-induced compromise of the immune system, and inadequate vaccine response. This is especially problematic because shelters and new adopters often lack the resources to complete the necessary treatment of respiratory infections. Thus, prevention of these respiratory infections could prevent the euthanasia of these otherwise adoptable puppies.

Research in humans has revealed that vitamin D modulates inflammation and immune response in respiratory tissue. Numerous studies have demonstrated that low levels of vitamin D in people lead to increased incidence and severity of a variety of respiratory diseases. Additionally, adequate levels of vitamin D correlate with decreased incidence of viral respiratory illness during the winter months. This research suggests that supplementation of vitamin D in deficient populations, such as puppies, may decrease their risk of developing respiratory diseases.

The proposed study will be divided into two parts. In part one, the serum 25-hydroxyvitamin D levels of two groups of owned puppies entering the Foster Hospital for Small Animals (FHSA) will be evaluated. One group of ten puppies will present to FHSA with signs of respiratory disease, and the second group of ten puppies will present without symptoms. The serum 25-hydroxyvitamin D levels will then be compared between the two groups. It is hypothesized that puppies with naturally occurring respiratory infections with have lower 25-hydroxyvitamin D than healthy puppies.

In the second part of the study, the vitamin D levels of thirty puppies admitted to a shelter will be determined. The puppies will then be tracked and separated into two groups depending on whether or not they develop respiratory infections. The vitamin D levels between the two groups will be compared. It is hypothesized that in puppies admitted to a shelter, those that develop symptoms of respiratory infections will have a lower 25-hydroxyvitamin D than those that do not become symptomatic.

If positive results are obtained in the proposed study, further work could ultimately investigate the use of vitamin D as a nutritional supplement to reduce the occurrence of respiratory infections in puppies.

Awardee: Rebecca Falender V'16

Mentor: Dr. Lois Wetmore

Award Type: NIH

Research Project: Effects of Methadone and Hydromorphone on Peri-anesthetic Gastroesophageal Reflux, Regurgitation, and Vomiting

Summary: In this study, we will measure the incidence of perioperative gastroesophageal reflux (GER), regurgitation, and vomiting in dogs pre-medicated with either hydromorphone or methadone, prior to undergoing surgery for cruciate repair (tibial plateau leveling osteotomy, lateral suture repair, or tibial tuberosity advancement).

The experiment will be a randomized, controlled study of 50 dogs undergoing elective orthopedic surgery at the Foster Hospital for Small Animals at Tufts Cummings School of Veterinary Medicine. Dogs enrolled in the study will be randomly selected to receive acepromazine with either hydromorphone or methadone (with or without an anticholinergic as determined by the patient's pre-anesthetic heart rate) as their pre-anesthetic drug protocol. The patients will be observed during the period between premedication and induction of anesthesia for signs of regurgitation and vomiting. They will be induced with propofol and maintained during surgery with isoflurane and oxygen. A sensor tipped catheter will be placed following intubation to measure the esophageal pH of the patient during the surgery to assess for the presence of reflux and they will be monitored for evidence of regurgitation. The patient will also be monitored for any signs of regurgitation or vomiting during their 2-3 days at the hospital. One month post-surgery, the client will be called to ascertain if any further vomiting, regurgitation or signs of esophagitis or aspiration pneumonia were observed.

During anesthesia, GER has been shown to occur in 17-60% of cases and, in a smaller number of dogs, is also associated with regurgitation.1 Previous studies have determined that morphine increases the incidence of GER and regurgitation; however, hydromorphone and methadone have never been studied. Pre-anesthetic opioids are a critical part of surgical pain management. Although regurgitation and vomiting has been observed for some time in association with the use of these drugs, the exact rate of occurrence with either drug has never been determined. GER from anesthetic agents may result in post-anesthetic esophagitis, stricture formation, and, in the case of regurgitation, aspiration pneumonia.2 We hope to identify a preferred pre-anesthetic opioid to minimize the incidence of GER and regurgitation for dogs that require anesthesia in the future.

Awardee: Aliza Gentili-Lloyd V'15

Mentor: Dr. Claire Sharp

Award Type: Morris Animal Foundation

Research Project: Serial Quantitative Real-Time PCR to Evaluate Feline Herpesvirus-1 Epidemiology in Shelter Cats and Its Association with Upper Respiratory Tract Disease

Summary: Specific Aims: The primary objective of this study is to perform quantitative real-time PCR (qPCR) on serial conjunctival and oropharyngeal swabs to evaluate feline herpesvirus-1 (FHV-1) epidemiology in shelter cats and its association with upper respiratory tract disease (URTD). In addition to determining the prevalence of FHV-1 in symptomatic and asymptomatic cats, we aim to compare our results with those previously determined by a commercially available qPCR. We aim to correlate the amount of FHV-1 virus shed over time with the development and severity of clinical signs of URTD during the cat's shelter stay, and how this compares to their antibody titers against FHV-1 at the same time points. Additional aims are to determine whether vaccination with a modified live FHV-1 strain results in detectable FHV-1 DNA in conjunctival or oropharyngeal swabs which may confound diagnostic results; and whether or not more information can be gained by quantifying virus separately from conjunctival and oropharyngeal swabs.

Methods: This study will use banked conjunctival and oropharyngeal swab samples from 99 cats collected by our research group between June 27 and August 19 2012 at the MSPCA Shelter in Methuen, MA. Swab DNA will be extracted using the Qiagen QIAamp DNA Mini Kit and eluted in a minimum volume. Extracted DNA will then be used for absolute quantitative PCR. Our assay will specifically amplify a small conserved region of the glycoprotein B gene of FHV-1 for absolute quantification of FHV-1 copy number in our experimental samples using a plasmid standard curve. Additionally we will amplify a small region of a feline reference gene (MC1R) to normalize for differences in the quantity of cells collected from swabs.

Significance: URTD is a treatable disease that commonly leads to euthanasia of otherwise healthy cats in shelters due to its highly contagious and difficult to manage nature. FHV-1 is one of the major causes of URTD, and poses a particular challenge because of its ability to cause latent and chronic infections, which can be reactivated during a stressful event. The exact role of FHV-1 as a causative agent of URTD in the shelter setting has been difficult to decipher due to its transient and intermittent shedding pattern. By quantifying FHV-1 over a series of time points for the 99 cats in our study, we will gain a more accurate representation of FHV-1 shedding in shelters. This will allow us to correlate the quantity of FHV-1 shedding to the serum antibody titers, as well as the incidence and severity of URTD at those same time points. Ultimately this research will provide essential information for a more evidence-based management strategy for URTD in shelters.

Awardee: James German V'16

Mentor: Dr. Christopher Schonhoff

Award Type: NIH

Research Project: Opposing Effects of cAMP and NO on NTCP Function

Summary: Cholestasis is a condition of impaired bile flow that can have serious health issues associated with it. Bile formation, which is decreased in cholestasis, is regulated by several membrane-bound transport proteins. Among these transporters, NTCP (Na+-coupled taurocholate transporting polypeptide) mediates uptake of bile acids into hepatocytes. The translocation of NTCP from intracellular stores to the plasma membrane has been found to be stimulated by cAMP. Conversely, previous research points to an inhibitory effect of nitric oxide on translocation of NTCP. In an attempt to highlight the interplay between cAMP and NO, this study will use a human hepatoma cell line that expresses NTCP and expose them to both molecules. After exposure, the effects on translocation of the NTCP transporter and the uptake of the bile acid, taurocholate, will be measured. Taurocholate uptake will be measured by assessing the uptake of radioactively labeled taurocholate into HuH-NTCP cells. The translocation of NTCP to and from the plasma membrane will be measured by selectively biotinylating plasma membrane bound proteins, purifying those proteins with streptavidin beads, followed by immunoblot for NTCP. It is believed that NO will have an inhibitory effect on the translocation of NTCP and the uptake of taurocholate. The goal of this research is to better understand the pathways involved with bile acid transport and the medical condition of cholestasis. More effective treatments may one day be developed from the insight gained through this study.

Awardee: Nida Intarapanich V'16

Mentor: Dr. Emily McCobb and Dr. Elizabeth Rozanski

Award Type: Merial Scholars Program

Research Project: Characterization and Comparison of Injuries caused by Accidental and Non-Accidental Blunt Force Trauma

Summary In cases of animal abuse, the actual cause of injury often differs from the description provided by the client. Determining the true nature of the incident is important for the treatment of the animal; furthermore, due to the well-supported correlation between animal abuse and domestic violence, child abuse, and elder abuse, identifying animal abuse and alerting appropriate authorities is essential for the safety and welfare of the animal and all members of the household.

Current guidelines to identify possible cases of animal abuse are based mostly on suspicious behavior in

the client or patient. There is a lack of in-depth studies of specific injuries caused by abuse; in particular, no guidelines exist to differentiate motor vehicle accidents and other accidental blunt force trauma from non-accidental injury. This retrospective study will examine cases of known motor vehicle accidents in dogs and cats and compare the presence, location, and pattern of rib fractures with cases of known abuse.

Cases of motor vehicle accidents (MVA) will be selected from the Foster Hospital for Small Animals at the Cummings School of Veterinary Medicine, and cases of non-accidental injury (NAI) from the ASPCA in New York City. Data collected will include: if rib fractures are present, whether fractures are present on one or both sides of the body, the total number of fractures, the average number of fractures per rib, the number of fractures on each specific rib, whether old fractures are present, which other bones are fractured, and if other conditions such as pneumothorax, diaphragmatic hernia, pulmonary contusions, or surface abrasions are present. We will look for statistically significant relationships between various factors and the cause of injury (MVA vs. NAI) as well as for patterns that may aid in distinguishing MVA from NAI. Statistics to be used include the chi-square test of independence, the t-test for two independent samples, factor analysis, and discriminant analysis.

Our goal is to add to the body of literature that aids clinicians in differentiating causes of trauma. Furthermore, evidence-based patterns of non-accidental injury for veterinary patients will aid in the documentation and potential prosecution of animal abuse.

Awardee: Jesse Lane V'15

Mentor: Seana Dowling, MS and Dr. Emily McCobb

Award Type: NIH

Research Project: Stress in Shelter Dogs: Use of a Behavioral Stress Ethogram to Evaluate the Relationship between Behavior and Urinary and Salivary Cortisol Levels and Activity Levels

Summary: Stress in dogs in animal shelters has been proven to cause an increase in disease and frequency of aggressive behaviors, decrease in adoption rates, and has obvious negative implications to welfare (Hennessy 1997). Between May and July of 2009, data was collected in a pilot study on 13 dogs kenneled at the Boston Animal Rescue League. This included video recordings of the dogs in the kennels the day after being admitted to the rescue as well as urinary and salivary cortisol levels and activity levels. *The goal of this summer is to use this data to perform a retrospective, observational study to construct a canine behavior stress ethogram and to use these behaviors to test the hypothesis that dogs displaying stress behaviors will have an increase in urinary and salivary cortisol levels and activity levels.*

This research could provide animal shelters with a means to practically recognize and evaluate stress in dogs. Further steps could then be taken to implement appropriate enrichment and behavioral

modifications and improve welfare. Such a resource could also have implications not only in animal rescues and shelters but also in animal hospitals, laboratories, and in the home.

Awardee: Katherine Leonard V'16

Mentor: Dr. Michael Kowaleski

Award Type: NIH

Research Project: Combined Tibial Plateau Leveling Osteotomy and Tibial Tuberosity Transposition for Treatment of Cranial Cruciate Ligament Rupture and Medial Luxating Patella

Summary The goal of this study is to better understand the outcome of surgical treatment for medial patellar luxation with concomitant cranial cruciate ligament rupture in canine patients. Individually, these two injuries are extremely common in veterinary practice and represent a major reason why many patients undergo orthopedic surgery. Recent research has suggested that in some dogs the two may be related and with this new information comes a need to investigate a successful means of treatment. A single surgical technique has yet to be shown more effective over others for treatment of this specific injury and by evaluating the outcome of this procedure, future studies may be conducted to compare its success to other procedures.

The surgical technique will be described and postoperative healing will be evaluated based on radiographs taken at subsequent recheck examinations, medical records and data collected from patient owners. The time of the postoperative radiographs will be recorded and healing outcome will be evaluated based on the International Society of Limb Salvage grading criteria. Owners will be contacted to obtain more information about lameness, exercise tolerance, and overall improvement observed. This information will be compiled and examined for trends in postoperative healing. Patient cases will be obtained from the veterinary teaching hospitals of Tufts Cummings School of Veterinary Medicine and Texas A&M College of Veterinary Medicine.

Awardee: Evin Luehrs V'15

Mentor: Dr. Esther Schelling, Dr. Michael McGuill and Dr. Joann Lindenmayer

Award Type: NIH

Research Project: Prediction Model of Livestock to Human Brucellosis Transmission Mongolia

Summary: Brucellosis is one of the most common infectious diseases of livestock and zoonotic diseases in the world. It is well controlled in industrialized countries, but is still prevalent in developing countries. In animals, brucellosis affects reproduction and fertility, resulting in economic losses to herders. In humans, it often causes chronic disease. Mongolia has seen a reemergence of brucellosis after the end of the socialist period and the shift away from dependence on the former Soviet Union in 1990. A government funded vaccination campaign has been ongoing in Mongolia since 2000 and currently has the target of brucellosis elimination by 2015. Previous research has found that 90% of human brucellosis was small ruminant derived. Continued success of the vaccination campaign depends on surveillance and monitoring. Because vaccinated animals will be seropositive for a long time after vaccination, the effectiveness of the vaccination campaign cannot be assessed by determining changes in the apparent seroprevalence in vaccinated livestock. However, it can be assessed indirectly using human seroprevalence data as an indicator of the status of animal infection. Having updated models of livestock to human transmission of brucellosis is critical for informing vaccination methodology.

The first aspect of the study will take place at the Swiss Tropical and Public Health Institute in Basel, Switzerland, and will include extensive review of the literature on human and livestock brucellosis seroprevalence in Mongolia in order to develop a preliminary prediction model. The second aspect of the project will take place in Mongolia and will involve interviewing brucellosis experts and collecting additional data in order testing the prediction model as part of the ongoing "Animal Health Project" run by the Swiss Agency for Development and Cooperation (SDC).

The objective of this study is to assess associations between seroprevalence in humans and livestock and independent variables including geographical region (defined at the provincial level), livestock species (cattle, sheep, goats), and human age and sex, in the transmission of brucellosis from animals to humans.

Awardee: Elissa Mopper V'15

Mentor: Dr. Giovanni Widmer

Award Type: NIH

Research Project: Epidemiology of *Cryptosporidium P*arasistes in Dairy Calves and HIV Patients in Two Locations in Thailand

Summary: *Cryptosporidium parvum* has long been known as a significant zoonotic protozoan parasite and a cause of potentially life threatening gastroenteritis in immunocompromised humans.1 Through the use of genotyping, we have come to discover certain *C. parvum* genotypes that appear to be adapted specifically to human hosts, lacking the ability to infect animals. In particular, a genotype of *C. parvum* termed IIc has exclusively been isolated from humans, with the minor exception of 5 isolates originating from hedgehogs in Germany.2 We hypothesize that *C. parvum* IIc may actually be a zoonotic

pathogen. This hypothesis is based on the fact that most *C.parvum* genotypes are zoonotic and that *C. parvum* IIc can be propagated in mice.3 We also hypothesize that animal infections with *C. parvum* IIc may have remained undetected because surveys of *C. parvum* in regions where IIc is common have not included livestock or have not determined the genotypes. Calves located in warmer climates, where *C. parvum* IIc is prevalent in humans,2 may provide the ideal host environment for determining whether or not *C. parvum* IIc infects animals in nature. Therefore, the purpose of this research is to determine whether *C.parvum* IIc is indeed anthroponotic, as currently assumed, or also infects animals. The study will focus on identifying dairy calves, in the Khon Kaen region of Thailand that are infected with *C. parvum* parasites. Oocysts will be collected from infected dairy calves and their DNA isolated to be shipped to TCSVM for genotyping. Additionally, we will also be analyzing fecal samples from HIV infected patients suffering from cryptosporidiosis, obtained from the Bamrasnaradura Infectious Diseases Institute located in Bangkok, in order to isolate oocyst DNA that will be used to determine the prevalence of *C. parvum* IIc in humans in Thailand. If the hypothesis is proven correct, i.e., IIc infects livestock, we will conclude that the assumption that *C. parvum* IIc does not infect animals is incorrect. The outcome of this project will assist in clarifying the epidemiology of cryptsporidiosis and elucidating the relative importance of anthroponotic and zoonotic transmission.

Awardee: Anya Price V'16

Mentor: Dr. Robert Bridges

Award Type: NIH

Research Project: Effects of Gestational Prolactin on Postpartum Anxiety and Maternal Behavior in Rats

Summary: Prolactin is a hormone of the anterior pituitary involved in mediating anxiety during pregnancy and the postpartum period. Bromocriptine, a dopamine agonist, is used to suppress circulating levels of prolactin. When administered to pregnant mice, it represses the postpartum maternal behaviors of the mice and results in elevated anxiety (Larsen & Grattan, 2010). A similar study on the repression of the anxiolytic effects of prolactin has not yet been performed in rats.

We hypothesize that female rats given bromocriptine during gestation will exhibit elevated anxiety, as well as decreased maternal behavior such as pup retrieval, especially when in a novel environment. The experiment should lead to a better understanding of the endocrine environment during gestation and its effects on postpartum behavior. This can lead to application in the field of human medicine regarding postpartum depression.

Awardee: Frank Romano V'16

Mentor: Dr. Cailin Heinze

Award Type: Merial Scholars Program

Research Project: Characterization of the Relationship Between Obesity and Cancer Progression in Dogs

Summary Evidence suggests that the high prevalence of both obesity and cancer in dogs may not be entirely separate events. Based on studies in humans and rodents, we have reason to believe that obesity is correlated with cancer in the form of increased risk, tumor aggressiveness, and chances of death. However, counteracting obesity is currently not a typical focus during cancer treatment in dogs, potentially because the relationship between obesity and cancer has not been suitably investigated in dogs. Therefore, we propose this study to help define this relationship; the demonstration of a relationship between an overweight body condition and the clinical course of cancer would benefit veterinary medicine in a variety of contexts. For example, nutritional intervention that alleviates obesity in cancer patients could potentially ameliorate cancer progression. Additionally, it may also be possible to reduce cancer risk in breeds predisposed to both cancer and obesity by providing early nutritional intervention. Thus, this investigation serves as a necessary initial step that may lead to further investigations of the underlying mechanisms that govern the role of obesity in the course of cancer, and eventually to therapeutic interventions that could combat this relationship. We will investigate the relationship between obesity and cancer survival in dogs through a retrospective study, utilizing medical records of previous lymphoma and osteosarcoma patients – two of the most common cancers in dogs. Using body condition scores and multiple cancer progression metrics as our variables of interest, we aim to define the relationships between the overweight body condition and both survival time and response to treatment for canine cancer patients.

Awardee: Stephanie Sapowicz V'15

Mentor: Dr. Deborah Linder

Award Type: Purina

Research Project: Prevalence of Obesity and Evaluation of Feeding Habits Across Various Pet Owner Incomes

Summary: At the Tufts Cummings School of Veterinary Medicine, students now have the unique opportunity to experience working at Tufts at Tech, a student-run veterinary clinic providing low-cost care for family pets within Central Massachusetts' underserved communities. The main objective of this summer student project is to gather descriptive data on pet obesity, feeding habits, and attitudes

towards pet food of the clientele at this low cost clinic to better prepare veterinarians to meet the nutritional needs of this population compared to the general population. The study has three specific aims: 1) to determine the prevalence of cat and dog obesity in varying income populations; 2) to determine the reasons for owners of various income brackets to feed commercial or non-commercial pet food; and 3) to determine if there is an association between income, obesity and owner feeding preferences. The study will include a control population to further increase the strength of the findings across various income levels. Based on the researchers' experiences at the clinic, our hypothesis is that obesity and non-commercial diets will be prevalent within the low income community, and owner preference of feeding non-commercial diets will be associated with obesity. While previous studies have investigated pet owners' attitudes towards obesity and pet foods in the varying pet populations, there is a need to further describe obesity and its relationship with owner attitudes and feeding habits within a low income population compared to a control general population. This descriptive data will allow veterinarians to better educate and treat patients from underserved communities, as well as highlight the need for optimal pet nutrition discussions with all clientele. On a larger scale, this data could provide the basis for educational and public health outreach within various communities.

Awardee: Stella Spears V'15

Mentor: Dr. Ben Nephew

Award Type: NIH

Research Project: Effects of Depression on Gene Expression in Adult Female Sprague-Dawley Rats

Summary: Depression is a serious mood disorder that disproportionately affects women twice as much as men. It is well known that there is a gender difference in the causes and progression of depression. In spite of this fact, the majority of depression research and drug development has been done using male models. Using a model more relevant to the female experience, research can instead be directed toward more applicable and personalized treatment.

The goal of this portion of the study is to determine whether there are changes in gene expression and methylation in those individuals affected by early life chronic social stress (CSS) as compared to those who have not undergone this stress. Any changes found can then be used for further study into the possible female-specific, chemical mechanism of depression and inform future preventative measures and treatment of depression.

Using brain samples from female rats that have undergone an ethological stress protocol to produce depression and control non-depressed animals, I will compare changes in gene expression and methylation. Previous study supports the importance of the chosen brain regions being included in this analysis. The chosen regions (paraventricular nuclei, supraoptic nuclei, central amygdala, medial

amygdala, lateral septum, periaqueductal gray matter, ventral tegmental area, anterior thalamus, and nucleus accumbens) are important in maternal behaviors and are likely to show a gender difference in depression. Analysis of mRNA expression will allow the quantification of differences in OXT, prolactin, CRH and AVP expression. Analysis of DNA methylation is used to monitor for possible epigenetic changes, as previous studies indicate that early life CSS induces stable long-term changes in gene expression.

Awardee: Carla Stoffel V'16

Mentor: Dr. David Sherman

Award Type: NIH

Research Project: Experimental Infection of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) to Evaluate Genetic Predisposition of Johne's Disease Among Different Breeds of Sheep in Australia

Summary: Objective: The main objective of this study is to evaluate whether susceptibility to *Map*, the etiologic agent of Johne's disease, has a genetic basis and is correlated to the breed of sheep. This study involves the experimental inoculation of four prevalent sheep breeds in Australia, followed by assessment of disease outcome at necropsy and collection of tissue specimens.

Animals: This study will be conducted in Camden, New South Wales, Australia, in conjunction with the ongoing Johne's research program at The University of Sydney. The lambs selected for this study were from four commonly used breeds of sheep in Australia and confirmed to be *Map*-free prior to inoculation. To confirm freedom from disease prior to inoculation and in control sheep, a pooled fecal culture test was used to assess the risk of infection. The status of Monitored Negative (MN) is allocated to a flock assessed as *Map*-negative, depending on how long a flock has participated in the program and therefore the number of times serological sampling has been undertaken, i.e. MN1, MN2 or MN3. All sheep were sourced from an MN3 flock.

Procedure: Researchers from The University of Sydney inoculated the lambs with a specific ovine strain of *Map* designed for experimental infection. By the time my portion of the project commences, those sheep from which the tissues are derived will have been necropsied and a full complement of tissues prepared for histological examination will be available, which will enable comprehensive analysis of gene expression affected by *Map*, taking into consideration the disease outcome i.e. whether the sheep were resistant to *Map* infection or had developed multibaciallary or paucibacillary disease. The QuantiGene ViewRNA ISH Tissue Assay will be used as a direct *in situ* hybridization method using target specific probe sets for detection of specific gene expression in response to *Map* exposure.

Relevance: Johne's disease is a contagious and untreatable disease of ruminants that leads to severe enteritis, wasting, and death of the infected animal. As such, the presence of *Map* has a significant herd health and financial impact to sheep flocks internationally, with current control methods focused on vaccination and eradication. Understanding differences in breed susceptibility to *Map* infection can help improve upon current disease control methods, thereby reducing the death rate and financial toll associated with *Map* and promoting the health of sheep flocks worldwide.

Awardee: Joseph Sweeney V'15

Mentor: Dr. Olivier Taeymans

Award Type: NIH

Research Project: A Novel Radiographic Measurement for Assessing Stifle Joint Instability in Dogs Affected with Cranial Cruciate Rupture

Summary: Background/Specific Aims: Cranial cruciate ligament (CCL) rupture is one of the most common causes of hind limb lameness in dogs, but can be hard to diagnose on physical exam at times. Additionally, clinicians most commonly rely solely on radiographic changes such as stifle joint effusion and peri-articular new bone formation to indirectly diagnose the condition. These secondary changes however are non-specific, and may be seen in other conditions affecting the canine stifle joint. The primary change of cranial tibial subluxation is sometimes noted on plain stifle radiographs of dogs affected with CCL rupture. Therefore, radiographically documenting tibial subluxation would further substantiate the diagnosis of this condition. Radiographic measurements have previously been reported for assessing cranial tibial subluxation, but due to their complexity, are rarely used in practice. This retrospective study is intended to establish a novel, quick, and simple-to-perform measurement that will objectively document radiographically visible subluxation. We further hypothesize that joints with a complete CCL tear will have significantly larger measurements than normal stifle joints. The study should help pave the way for future prospective studies evaluating the prevalence of spontaneous tibial subluxation on stifle radiographs of dogs with CCL, and may subsequently also be used in evaluating partial vs. complete CCL tears.

Experimental Design: This study will evaluate a radiographic measurement, retrospectively using 30 dogs with complete CCL rupture that have been confirmed at surgery. We will measure distances between landmarks on the femur and the tibia, and compare these measurements with a matched control population.

Awardee: Colleen Thurman V'16

Mentor: Dr. Sawkat Anwer

Award Type: NIH

Research Project: Role of p38MAPK in the Anti-cholestatic Effect of Tauroursodeoxycholate

Summary: Bile secretion is dependent on transport of solutes from blood to bile. Thus, transporters should be at the appropriate plasma membrane for bile secretion to occur. Failure to do so may result in cholestatic diseases. Taurolithocholate is known to cause cholestatic disease by removing the transporter multidrug resistant protein 2 (Mrp2) from the canalicular membrane, and it has been shown that tauroursodeoxycholate (TUDC) can reverse this removal and the accompanying cholestasis. P38MAPK is known to contribute to Mrp2 translocation through a cAMP dependent pathway. Thus, it is possible that TUDC may reverse TLC-induced Mrp2 retrieval and cholestasis by activating p38MAPK. The specific aim of this project is to test the hypothesis that TUDC translocates Mrp2 from the cytosol to the canalicular membrane by a p38MAPK dependent pathway. This hypothesis will be tested by answering two questions: 1) Does TUDC activate p38MAPK? And, if so 2) Does an inhibitor of p38MAPK inhibit TUDC-induced translocation of Mrp2?

Awardee: Danielle Woolf V'16

Mentor: Dr. David Sherman, Dr. Jean Mukherjee and Dr. Carlos Sanchez

Award Type: NIH

Research Project: Seroprevalence of *Leptospira* sp. in Non-Human Primates, Rats, and Squirrels at the Barranquilla Zoo, Colombia

Summary: Leptospirosis is the most widespread zoonotic disease. It is caused by pathogenic *Leptospira* sp. and is shed in urine of infected hosts and transmitted via direct or indirect contact with the host or contaminated environmental sites. Leptospirosis is currently endemic in Colombia, as the pathogen favors tropical climates and wet seasons. The seroprevalence within the rodent (rats, mice), dog and human population in Barranquilla, Colombia, is 57.9%, 22.9% and 12.4%, respectively. Rodents have been recognized to be the most important and widely distributed reservoirs of *leptospiral* infection. Recently, a survey of 17 species from four Colombian zoos revealed that the highest seroprevalence of leptospirosis was in non-human primates.5 The presence of leptospirosis in non-human primates at the Barranquilla Zoo is most likely due to free roaming rodents accessing their enclosures. We hypothesize that non-human primates resident at the Barranquilla Zoo are likely seropositive for *Leptospira* sp. and that synathropic rodents, including squirrels and rats are likely reservoirs for infection of the primates. A surveillance study testing rats,

squirrels and captive primates at the zoo for the presence of pathogenic *Leptospira* sp. will determine this. This will involve trapping the animals and collecting samples from them, which will then be analyzed in a local laboratory using microscopic agglutination test (MAT) for serodiagnosis, polymerase chain reaction (PCR) for detection of the pathogenic *Leptospira* gene, and pulsed-field gel electrophoresis (PFGE) for serovar identification. Infection of *Leptospira* sp. In captive primates would reveal access to their enclosures by rodents and thus their exposure to the pathogen. Pathogenic *Leptospira* serovars common to both species at the Zoo would indicate rodents as the source of infection.

Awardee: Siobhan Wright V'16

Mentor: Dr. Elizabeth Byrnes

Award Type: NIH

Research Project: The Effects of Non-Medical Opiate Use on Serotonin, Serotonin Receptors, and Serotonin Transporters of F1 and F2 Generation Rats in the Study of Maternal Aggression

Summary: The purpose of this study is to examine how non-medical opiate use affects neurotransmitters in generations of populations after initial adolescent exposure. This will be an expansion of transgenerational modifications of maternal aggression previously studied by the Byrnes laboratory using rats as models of adolescent opiate use. This study aims to find a possible link to alterations in neurodevelopment that could cause the transgenerational changes observed.

Having been previously linked to maternal aggression, this experiment will focus on levels of the monoamine serotonin (5-HT) and expression of 5-HT transporter (5HTT) mRNA, 5HT2a and 5HTlb receptors. These will be analyzed using high performance liquid chromatography and quantitative real time PCR from two groups of Fl and F2 rats that will be generated prior to the start of this study. One group will be exposed to morphine and the control groups will be exposed to saline. Subjects will be euthanized and portions of the brains associated with maternal aggression from each group will be frozen and processed. Statistical analyses will then be conducted in order to look for significant differences in the neurotransmitter systems known to regulate maternal aggression in Fl and F2 generations.

Serotonin agonists produce antidepressant and anxiolytic effects. Low 5-HT levels has been linked to defensive aggression, which has been suggested as characteristic of maternal aggression. Working with those associations in mind, this study expects to find that the rats in the F2 generation exposed to morphine, the experimental group that has shown an increase in maternal aggression, will exhibit the lowest levels of 5-HT metabolism or highest amount of serotonin receptors and serotonin transporters.

Awardee: Bushra Zaidi V'15

Mentor: Dr. Sawkat Anwer

Award Type: Merial Scholars Program

Research Project: Cyclic AMP Dependent Translocation of NTCP and MRP2 to Plasma Membrane of Hepatocytes Mediated by Phosphorylation of Thr505 of Protein Kinase C Delta

Summary: Bile formation requires transport of solutes from blood and secretion into bile by hepatocytes. Multiple transport proteins located at the basolateral and canalicular membrane accomplish this goal. Some of these transporters include Na^+-taurocholate cotransporting polypeptide (NTCP) at the basolateral membrane and multidrug resistant protein 2 (MRP2) at the canalicular membrane. Studies have suggested that cyclic adenosine monophosphate (cAMP) stimulates NTCP and MRP2 translocation by activating novel Protein Kinase C delta (nPKCδ).

The aim of the present study is to test the hypothesis that cAMP-induced translocation of NTCP and MRP2 involves activation of nPKCδ by phosphorylation of Thr^{505}. Two specific aims are proposed to test this hypothesis: 1. Determine the effect of dominant negative mutant of nPKCδ (nPKCδT505A) on cAMP-induced translocation of NTCP and MRP2. 2. Determine the effect of constitutively active phosphorylation mimicking mutant of nPKCδ (nPKCδT505D or nPKCδE) on cAMP-induced translocation of NTCP and MRP2.Dominant negative mutant of nPKCδ-Thr^{505} will be constructed via site-directed mutagenesis by replacing threonine to alanine (nPKCδ-T505A). Similarly, a constitutively active mutant will be constructed by replacing PKCδ-Thr^{505} to aspartic acid or glutamic acid (nPKCδ–T505D or nPKCδ–T505E).

A cell surface biotinylation method will be used to quantitate NTCP and MRP2 translocation after the mutant constructs are expressed in the HuH-NTCP cell line. Briefly, after various treatments, cells will be incubated with sulfo-NHS-LC-Biotin to achieve the selective labeling of cell surface proteins. Biotinylated proteins will be isolated with streptavidin-agarose beads followed by immunoblot analysis. The proposed studies should further define the mechanism by which nPKCδ mediates cAMP-induced translocation of NTCP and MRP2. The proposed studies should further define the mechanism by which nPKCδ mediates cAMP-induced translocation of NTCP and MRP2.